C. Environmental Assessment

Background

The Monsanto Company has requested a registration for *Bacillus thuringiensis* Cry3Bb1 protein and the genetic material (ZMIR13L) necessary for its production in all corn lines and varieties. The Cry3Bb1 protein is intended to control the corn rootworm (CRW, *Diabrotica* spp.), a coleopteran pest of corn. The CRW is a primary pest of corn in the U.S., it feeds on corn roots as larvae leading to a reduction in the plant's ability to absorb water and nutrients from soil and lodging (i.e., loss of structural integrity of the corn stalk). In areas where the CRW is a pest (e.g., Corn Belt), significant financial losses are realized from a decrease in production and chemical insecticide usage. Significant acres of corn are treated annually to control CRW with organophosphate, carbamate and pyrethroid insecticides.

In addition to the review of the final submission of the field sampling data by Monsanto, this section addresses comments and conclusions from the August 27, 2002 Scientific Advisory Panel (SAP) meeting titled *Corn Rootworm Plant-Incorporated Protectant Insect Resistance Management and Non-Target Insect Issues*. A memorandum dated November 6, 2002 from Paul I. Lewis to Marcia E. Mulkey included a transmittal of the meeting minutes of the November 27, SAP. Based on the SAP and public comments, the Agency is providing supplemental conclusions to those found in the May 20, 2002 preliminary review of ecological non-target insect studies for *Bacillus thuringiensis* Cry3Bb1 protein.

The Agency has conducted an environmental hazard assessment of the Cry3Bb1 producing corn lines. The general topics covered include gene flow to related wild plants, development of weediness, effects on wildlife, and fate of Cry3Bb1 proteins in the environment. The assessment is based on data submitted to the Agency during the development of the corn lines, additional data submitted for registration, FIFRA Scientific Advisory Panel (SAP) recommendations, consultations with scientific experts, and public comments received.

I. Non-Target Wildlife Hazard Assessment

A. The Hazard Assessment Process

The Agency assesses the toxicity of a Cry protein (*B.t.* endotoxin) to representatives of potentially exposed non-target organisms by a tiered testing system starting with Tier I single species maximum hazard dose laboratory data using mortality as the end point. This approach was developed for the Agency by the American Institute of Biological Sciences and approved in 1996 as an acceptable ecological hazard assessment method by a FIFRA Scientific Advisory Panel for microbial pesticides and microbial toxins, and by the December 9, 1999 SAP for protein Plant Incorporated Protectants (PIP). The methods were last published as the

Harmonized OPPTS Testing Guidelines (EPA 712-C-96-280, February 1996). The guidelines include (but are not limited to) bacteria and their toxins as defined in 40 CFR 152.20. The guidelines apply to microbes and microbial toxins when used as pesticides, including both those that are naturally occurring, and those that are strain-improved, either by natural selection or by deliberate genetic manipulation.

The guidelines in Tier I reflect a maximum hazard approach to testing. Negative results from tests using this approach provide a high degree of confidence that no unreasonable adverse effects are likely to occur. The OPPTS Harmonized Testing Guidelines utilize the tier testing scheme to ensure, to the greatest extent possible, that only the minimum data sufficient to make scientifically sound regulatory decisions will be required. Moreover, the Agency believes that the Tier I maximum hazard dose testing requirement represents a reasonable approach to evaluating hazard related to the use of biological pesticides, and is one in which negative results allow a high degree of confidence in the safety of the test agents. The Agency expects that most of the plant incorporated Bt Cry proteins require testing only in the first tier for short term hazard assessment. Long range adverse effects have to be ascertained by higher tier long term field testing. A SAP convened in October 2000 and the National Academy of Sciences (NAS 2000) also recommended testing non-target organisms directly in the field. This approach, together with an emphasis on testing of invertebrates found in the corn fields was also recommended by the August, 2002 SAP, and was supported by several public comments.

The maximum hazard dose approach for Tier I testing is based on a safety factor times the maximum amount of active ingredient expected to be available to terrestrial and aquatic plants and animals in the environment (the expected environmental concentration, or EEC). Therefore, data that establishes an LC_{50}, ED_{50}, or LD_{50} that is greater than the maximum hazard dosage level (e.g. LD_{50} >10 x EEC) is sufficient to evaluate adverse effects. If there are no effects at the maximum hazard dose, lower dose testing is not necessary.

If, however, significant toxic effects are noted at the maximum hazard dose level, the Harmonized Guidelines call for testing of multiple groups at lower doses in order to quantify the hazard. Sufficient doses and test organisms are required to determine an LD_{50} value and, if necessary, the No Observed Effect Level (NOEL), or reproductive and behavioral effects such as feeding inhibition, weight loss, etc. are evaluated. Appropriate statistical methods are to be used to express trends and to evaluate the significance of differences in data obtained from different test groups. The statistical methods used must reflect the current state-of-the-art with appropriate statistical power.

The Guidelines call for testing of a single group or groups of test animals at the maximum hazard dose, and if deleterious effects are observed, testing with sequentially lower doses to establish a definitive LD_{50} with confidence limits. When the active ingredient is a toxin, the appropriate endpoint would be death of the test organism. Each treatment and control group shall contain at least 10 test animals. When there is only one treatment group, at least 30 animals must be tested at that treatment level. The guidelines provide that the duration of all Tier I tests be about 30 days long. Some test species, notably non-target insects, may be difficult to culture and the test duration has been adjusted accordingly. Control and treated insects should be observed for a duration of at least 30 days after dosing, or in cases where an insect species cannot be cultured for 30 days, until control mortality rises above 20 percent.

On December 9, 1999, the Agency presented the maximum hazard dosing approach to testing of protein PIP and for possible new data requirements to a FIFRA Scientific Advisory Panel for their recommendations. The December 1999 SAP was generally supportive of the Agency's testing and hazard evaluation. The Panel also recommended more testing of non-target invertebrates more closely related to the target species and species more likely to be present in the field of the GM crops. In addition, the October 2000 SAP recommended appropriate field testing be conducted for non-target organisms. The August, 2002 SAP and certain public comments also agreed with this approach with some additions. It was recommended that the choice of appropriate indicator organisms for testing be based on the potential field exposure as deduced from data on Cry protein activity and expression in the plant. The SAP thought that appropriately chosen single species Tier I laboratory tests showing no detrimental effects are sufficient to make a short term hazard assessment and that field studies be conducted when these tests show toxicity (as higher Tier testing described in the OPPTS Microbial Testing Guidelines) but that proper multi-year commercial field studies with appropriate statistical power are needed to determine long term ecological effects. The December 9, 1999, SAP, the August, 2002 SAP, and several public comments noted that the maximum hazard approach to non-target species testing was not statistically appropriate for determination of No Observed Effect Levels (NOEL). This comment is in agreement with the Agency's OPPTS Testing Guideline discussed above.

Bt Cry endotoxins are proteins and, unlike inorganic chemicals, do not have the potential to bioaccumulate and thereby result in delayed effects. An accumulation through the food chain is therefore not expected to take place, and there are no data to support this possibility for protein substances. The basic biological properties of proteins also make Bt Cry proteins readily susceptible to metabolic, microbial, and abiotic degradation once they are ingested or excreted into the environment. Although there are reports of soil binding under certain circumstances, the bound Cry proteins are also reported to be rapidly degraded by microbes upon elution from soil. The same sources also report that Bt proteins in the soil of Bt corn fields have no detectable effect on soil invertebrates or culturable microbial flora. In addition, Bt Cry proteins do not have any characteristics in common with persistent, bioaccumulative chemicals that are transferred through the food chain. Therefore, chronic effects testing of protein substances is not routinely performed.

B. Cry3Bb1 Protein Environmental Hazard Assessment

Introduction

Based on the evaluation of the submitted maximum hazard dose testing data and information on the general biology of Bt Cry proteins, no unreasonable adverse effects on the invertebrate fauna of the corn field are expected from Cry3Bb1 protein producing corn. EPA concludes that it is appropriate for long term environmental effects to be assessed by appropriately designed field monitoring during the initial years of the Cry3Bb1 corn registration. EPA believes that the development and review of such information will also address one of the major concerns in several public comments.

Specific data are cited relating to aquatic and terrestrial wildlife, Cry protein fate in soils, potential effects on soil biota represented by the earthworm and field census data, effects on non-target soil Coleoptera species, foliar insects and endangered or threatened species, particularly Coleoptera. The results of these studies are presented here in both tabular (Table 1) and more detailed descriptive format. The complete review record of the submitted data can be found in the individual Data Evaluation Reports (DERs).

1. Summary of Non-Target Organism Toxicity Testing

Two separate SAP reports (October, 2000 and August, 2002) recommended that non-target testing be focused primarily on species exposed to the crop being registered. However, in addition to testing species directly exposed to the CryBb1 protein in the field, the full battery of non-target wildlife species testing was conducted to comply with the published Agency non-target data requirements for microbial toxins. (In the absence of PIP-specific data requirements, EPA requires applicants for PIP registrations to meet the 40 CFR. Part 158 data requirements for microbial toxins.) The Agency has determined that the non-target organisms most likely to be exposed to the protein in transgenic corn fields were beneficial insects feeding on corn pollen and nectar, and soil invertebrates, particularly Coleoptera ssp. Initially, in lieu of extensive and difficult single species laboratory soil coleopteran toxicity testing followed by an extrapolation to community risk assessment, direct field census data, and data on coleopteran insect effects and abundance in the field were requested, received and evaluated. The August, 2002 SAP, however, found the field census data unsatisfactory because of low statistical power. Maximum hazard dose toxicity testing on representative beneficials from several taxa were also performed. The toxicity of the Cry3Bb1 protein has been evaluated following challenge of several species of invertebrates including: adult and larval honey bees, a parasitic hymenopteran (*Nasonia*), green lacewings, lady beetles, collembola, monarch butterfly, and earthworms. Reproductive and developmental observations were also made on collembola, honeybee and lady beetle larva maturation studies. The August, 2002 SAP (as well as several public comments) however, found the green lacewing and parasitic wasp studies lacking and recommended testing of alternative species. Based on worst-case soil concentration, soil degradation studies show that Cry Bb1 protein in corn tissue is no longer detectable in agricultural field soil after 22 to 28 days. The August, 2002 SAP (as well as several public comments), however, suggested that additional soil degradation testing is desirable in a larger variety of soils and climactic conditions.

The non-target organisms tested are chosen as representative indicators of the major groups of wildlife and on the potential for field exposure as deduced from data on Cry3Bb1 protein expression in the plant. Although Bt Cry proteins are very specific in their activity to only certain insect species, for Cry3Bb1 protein in corn the Agency has examined the toxicity to birds, fish, honeybees and certain other beneficial insects even though a recent SAP (October, 2000) recommended against testing of non-targets species not related to those susceptible to the specific activity of Bt Cry proteins. However, in order to comply with the published Agency data requirements (40CFR Part 158) for registration of microbial toxins, the Agency asked for avian and aquatic invertebrate toxicity data, as well as Collembola (springtail) and earthworm species to ascertain effects on beneficial soil invertebrates because prolonged exposure to Cry3Bb1 proteins in soil was a possibility. Earthworm studies were also conducted and voluntarily submitted to the Agency by the registrant to demonstrate a lack of MON 863 effects on beneficial decomposers. Honeybee effects on brood as well as adults were required as exposure to the Cry3Bb1 protein in pollen is expected.

The form of the test substances used in the studies for this assessment are plant material such as leaves, roots, pollen or purified bacterially-produced Cry3Bb1 protein incorporated into the test species diet. The October 2000 SAP provided guidance to the Agency that while actual plant material is the preferred test material, bacterially-derived protein is also a valid test substance, especially in testing where the test animals do not consume corn plant tissue and where large amounts of Cry protein are needed for maximum hazard dose testing. As per the OPPTS Harmonized Testing Guidelines, the adult insect studies were generally of 30 days duration or until the negative control mortality reached 20%. Larval studies were through pupation and adult emergence.

Table 1. Tabular results of non-target wildlife and soil fate studies

Guideline No	Study	Results	MRID No.
USEPA OPPTS 885.4150	Wild Mammal Testing, Tier I	Mammalian wildlife exposure to Cry3Bb1 protein is considered likely; however, the Cry3Bb1 toxicity data for Human Health Assessment indicate that there is no significant toxicity to rodents from testing at the maximum hazard dose. Therefore no hazard to mammalian wildlife is anticipated.	Not assigned
885.405	A Dietary Toxicity Study with the Northern Bobwhite	The dietary LC_{50} value for Cry3Bb1 corn grain to juvenile Northern Bobwhite was greater than 70,000 ppm (10% of the diet) in a 8-day study (eight day observation). No adverse effects on avian wildlife is expected from incidental field exposure to Cry3Bb1 corn. A higher corn concentration and longer duration broiler study with MON863 corn is recommended. Classification: Supplemental.	449043-15

Guideline No	Study	Results	MRID No.
885.42	Freshwater Fish Testing	No treatment mortality or behavior change was observed among channel catfish in an 8 week sub-chronic study when fed diets containing 35% Cry3Bb1 corn lines MON 853 and MON 859. Classification: Acceptable.	449043-19
885.42	Freshwater Fish Testing	The requirement for a fresh water fish static renewal rainbow trout toxicity study has been waived based on a lack of any substantial exposure of fish to the Cry3Bb1proteins produced in corn crops.	Not assigned
Series 72, Subdivi-sion E	Acute Toxicity Test with the Cladoceran (*Daphnia magna*)	The 48-hour LC50 value for Cry3Bb1 corn pollen when administered to neonate daphnids was >120 mg pollen/L, a maximum hazard dose. No other adverse effects were noted. Therefore, no hazard to daphnia are expected from incidental exposure to Cry3Bb1-containing corn pollen. Classification: Acceptable.	449043-18
885.428	Estuarine and Marine Animal testing, Tier I	The Estuarine and Marine animal studies are waived for this product because of very low to no potential for exposure to Cry3Bb1 protein from field corn.	Not assigned
885.43	Nontarget Plant Studies, Tier I	Since the active ingredient in this product is an insect toxin (Bt endotoxin) that has never shown any toxicity to aquatic or terrestrial plants, these studies have been waived for this product. Outcrossing issues are addressed below.	Not assigned
885.438	Honey Bee Larva Testing	The LC_{50} for honeybee larvae and maturation to adult bees was determined to be >1,790 ppm Cry3Bb1 protein, (100X the concentration in pollen)in a maximum hazard dose study. Therefore no hazard to honeybee larvae and adult bee emergence is anticipated. Classification: Acceptable.	449043-10
885.438	Adult Honey Bee Testing	An adult honeybee maximum hazard dose feeding study showed the LC_{50} of the Cry3Bb1 protein to be >360µg/mL. (20X the concentration found in pollen). Therefore, no hazard from the Cry3Bb1 protein to honeybees is expected. Classification: Acceptable.	449043-11
885.434	Parasitic Hymenoptera Larva Testing	The LC_{50} for parasitic Hymenoptera was determined to be >400 ppm Cry3Bb1 protein. Although 400 ppm Cry3Bb1 protein is only 1X field concentration in plants rather than 10X, parasitic Hymenoptera are not expected to feed directly on corn plant tissue. Therefore, minimal exposure and no hazard to parasitic Hymenoptera from Cry3Bb1 protein is expected. Testing of a species more common to corn fields is recommended. Classification: Acceptable.	449043-13

Guideline No	Study	Results	MRID No.
885.434	A Dietary Toxicity Study with Green Lacewing Larvae	The LC_{50} for green lacewing larvae was determined to be >8,000 ppm Cry3Bb1 protein (20X field exposure). Based on these results it can be concluded that green lacewing will not be adversely affected when exposed to Cry3Bb1 in the field. Because of questionable ingestion of the test material another species (e.g, minute pirate bug, predatory carabid) more likely to be exposed to Cry3Bb1 should be tested. Classification: Supplemental.	449043-12
885.434	Effects of Bt Protein 11231 on Adult Lady Beetles *(H. convergens)*	This maximum hazard dose study showed that the LC_{50} for Cry3Bb1when fed to adult *H. convergens* is >8,000 µg purified Bt protein/mL diet., equivalent to 20X the maximum Bt protein concentration in plant tissue. A follow-up pollen feeding study was requested. Classification: Acceptable.	449043-14
885.434	Lady Beetle Larval Pollen Feeding Study (*C. maculata*)	The LC_{50} for Cry3Bb1 expressed in pollen is >93 µg/g fresh pollen weight. The larvae were observed through pupation to adult emergence. It can be concluded from this study that *Coleomegilla maculata* larvae will not be adversely affected by Cry3Bb1 field corn pollen. Classification: Acceptable.	455382-04
885.434	Adult Lady Beetle Pollen Feeding Study *(C. maculata)*	No significant adverse effects were noted in a 30 day 50% pollen feeding study. Based on these results, no hazard to *Coleomegilla maculata* is expected when feeding on Cry3Bb1 corn pollen in the field. Classification: Acceptable.	453613-01
885.434	Adult Lady Beetle Pollen Feeding Study (*H. convergens)*	No significant adverse effects were noted in a 15 day 50% pollen in honey water feeding study. Based on these results , no hazard to *Hippodamia convergens* is expected if feeding on Cry3Bb1corn pollen in the field. Classification: Acceptable.	453613-02
885.434	Collembola Chronic Dietary Toxicity Study	The LC_{50} of the Cry3Bb1 protein for Collembola was found to be >872.5µg (50% corn leaf tissue in the diet). No adverse reproductive effects were noted. It can be concluded from this test that Cry3Bb1protein does not pose a hazard to Collembola, a representative of a beneficial decomposer soil inhabiting species. Classification: Acceptable.	449043-17
850.62	Earthworm Toxicity Study	A maximum hazard dose 14-day LC_{50} for earthworms exposed to Cry3Bb1 protein in an artificial soil substrate was determined to be > 570 mg Cry3Bb1 protein/kg dry soil, or greater than 10 times the maximum EEC of the protein. The data show that no adverse effects to earthworms are expected from exposure to Cry3Bb1 protein producing corn plants. Classification: Supplemental.	449043-16

Guideline No	Study	Results	MRID No.
OECD Guideline 207	Earthworm Toxicity Study	There were no earthworm mortalities or other remarkable observations during the 14 day study. The LC_{50} value is greater than the highest maximum hazard concentration tested [166.6 mg Cry3Bb1 protein variant 11098 (Q349R)/kg dry soil].Classification: Acceptable.	457571-01
885.434	Monarch Butterfly Larval Pollen Feeding Study	This study has demonstrated that corn pollen expressing the Cry3Bb1 protein will not result in acute toxic or developmental effects to monarch larvae. The SAP recommended testing *Tetraopes* (red milkweed) beetles as a more logical choice than the monarch butterfly. Classification: Supplemental.	455382-05
N/A	Insecticidal Activity Spectrum study	Bioassays of six Families of the Order Coleoptera and two Lepidoptera species detected activity only against beetle species of the family Chrysomelidae (corn rootworm and Colorado potato beetle). Classification: Supplemental.	455328-07
154-3500	Field evaluation of Cry3Bb1corn exposure on non-target organisms	Preliminary results from two year Tier IV field census studies. These studies are supplemental to Tier I maximum hazard dose testing. The data do not show any MON 863 corn related adverse effect on non-target and beneficial invertebrate abundance in the field. Classification: Supplemental.	455382-06
154-3500	Non-target organism field scale risk assessment	Final report for MRID 455382-06 two year field census study. MON863 showed no overall differences in the abundance of non-target invertebrates and had less impact on certain beneficial insects compared to traditional insecticides, especially soil and foliar applications. These studies are supplemental to Tier I maximum hazard dose testing and are of inadequate statistical power for long term effects determination. Classification: Supplemental.	457916-01
154-3500	Field and Laboratory invertebrate studies	Summary (without data) of preliminary findings from several one year supplemental higher Tier field and laboratory studies not triggered by Tier I maximum hazard dose testing data. Final report of studies to be submitted. Classification: Supplemental.	456530-03
885.52	Aerobic Soil Degradation of the Cry3Bb1 Protein 11098	Finely ground corn leaf tissue in sandy loam field soil degradation data at worst-case field concentrations show that the Cry3Bb1 protein DT_{50} based on insect bioassays and ELISA were 2.37 and 2.76 days respectively. The DT_{90} estimates for the insect bioassays and ELISA were 7.87 and 9.16 days respectively. At $\leq$28 days the CryBb1 protein was below the detection level. These results verify that the Cry3Bb1 protein degrades rapidly and does not accumulate in the soil. Additional testing in different soil types is requested. Classification: Supplemental.	451568-04

Guideline No	Study	Results	MRID No.
885.52	Aerobic Soil Degradation of Cry3Bb1 Produced by CRW Protected Corn Event MON 863	The DT_{50} values for Cry3Bb1in several dosing regimes and soil types ranged from 0.6 days to 2.3 days and the DT_{90} values ranged from 4.03 days to 50 days. Cry3Bb1 levels in soil sample extracts show that concentrations were near or below the ELISA LOQ (0.16 µg/g) after 2 months of incubation. Additional studies with whole plant tissue are requested. Classification: Supplemental.	457571-02
None	Endangered Species Impact Assessment	Monsanto conducted a hazard assessment, exposure assessment and risk characterization to demonstrate that Cry3Bb1 does not pose a risk to endangered Coleoptera. The Agency performed an independent assessment and determined that no adverse affects from Cry3Bb1event MON 863 are expected to endangered/threatened Coleoptera species listed by the USFWS. Non-coleopteran endangered species are not expected to be susceptible to MON 863.	455770-03

2. Non-target Wildlife Testing and Hazard Assessment

a. Mammalian Wildlife

Mammalian wildlife exposure to Cry3Bb1 protein is considered likely; however, the mammalian toxicology information gathered to date on Bt Cry proteins does not show a hazard to wild or domesticated mammals. The Cry3Bb1 toxicity data for Human Health Assessment indicate that there is no significant toxicity to rodents from acute oral testing at the maximum hazard dose. Therefore no hazard to mammalian wildlife is anticipated.

b. Avian hazard assessment

The methods used in conducting the study (MRID No. 449043-15) followed procedures specified in EPA's Registration Guidelines, Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms; OECD Guideline 205, Guideline for Testing of Chemicals, Avian Dietary Toxicity Test and on ASTM Standard E857-87, "Standard Practice of Conducting Subacute Dietary Toxicity Tests with Avian Species."

The dietary LC_{50} value for Cry3Bb1 corn grain (MON 853, MON 854 and MON 855) when fed to juvenile Northern Bobwhite for 5 days was reported to be greater than 70,000 ppm (10% of the diet), the only concentration tested. No adverse effects on bobwhite quail were seen in eight days. These data show that there will be no hazard to avian wildlife from incidental field exposure to Cry3Bb1 corn. These data are, however, not sufficient to make a hazard assessment from repeated exposure to higher doses of Cry3Bb1 corn. This study is classified as supplemental. The concentration tested (10% corn in the diet) is too low. A six week broiler study with 60% - 70% MON 863 corn in the diet is needed to assess hazard to non-target birds from continuous exposure to high levels of Cry3Bb1 protein.

c. Aquatic species testing

There is no evidence for sensitivity of aquatic (including endangered) species to Cry proteins. Toxicity studies with Cry proteins on aquatic organisms show no hazard for fish or invertebrates exposed to either corn pollen or to bacterially expressed Cry protein. In addition, aquatic exposure from Bt corn is extremely small. When a simple standard pond scenario (1 hectare pond, 2 meters deep draining a 10 hectare watershed planted with corn) was used to develop a worst case EEC for Cry3Bb1 protein on the basis of corn pollen loadings from airborne pollen deposition and agricultural runoff from corn plant tissue left in the field at the end of harvest (assuming that no degradation of the protein takes place), airborne and agricultural runoff is calculated to be 3.9 ng Cry3Bb1 protein/mL. Thus, total water concentration of less than 3.9 ng Cry3Bb1 protein/mL is projected under worst case conditions.

i. Freshwater fish

The Harmonized Testing Guidelines requirement for a static renewal freshwater fish toxicity study is usually waived based on low to nonexistent exposure to Cry protein produced in corn. Exposure from corn pollen, if it does take place, will be of a very short duration and quantity and is not expected to have any detectable effect on freshwater fish. Nevertheless a subchronic eight week farmed channel catfish feeding study was performed and submitted for review.

The procedures used in the catfish study (MRID No. 449043-19) follow those recommended by EPA Pesticide Testing Guidelines for Microbial and Biochemical Pest Control Agents, Subdivision M and OPPTS Series Guideline 885.4200 - Freshwater Fish Testing. The testing was done at the Thad Cochran National Warmwater Aquaculture Center (Testing Facility), Mississippi State University, Stoneville, MS. The study was conducted in compliance with the U.S. EPA FIFRA Good Laboratory Practic Regulations (40 CFR Part 160) with three minor deviations which had no impact on the integrity of the study.

The study is scientifically sound and no treatment mortality or behavior change was observed among channel catfish fed diets containing finely ground corn grain from two insect-protected Cry3Bb1 corn lines (MON 853 and MON 859) for eight weeks. The results indicate that corn grain derived from the two transgenic lines producing Cry3Bb1 can be used as a feed ingredient in channel catfish diets at levels of up to 35% without adverse effect on fish growth, feed conversion efficiency, survival, behavior, or body composition. Significant differences were observed only as lower percentage fillet moisture among fish fed corn grain of the line MON 859; however, these are relatively unremarkable and are unlikely related to the different diets. There were no significant differences noted in feed consumption, weight gain, feed conversion ratio, survival, percentage visceral fat, or percentages fat, protein, or ash in fillets of channel catfish fed the different test diets. No abnormal fish behavior was observed in the study.

In view of the lack of demonstrated toxicity to channel catfish and minimal aquatic exposure, no fresh water fish hazard is expected from the uses of Cry3Bb1 protein in corn crops.

ii. Aquatic invertebrates

This study (MRID No. 449043-18) was conducted according to procedures specified in Series 72 of EPA's Registration Guidelines, Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation for acute toxicity testing of pesticidal substances to freshwater aquatic invertebrates.

The study was performed on *Daphnia magna*, a freshwater invertebrate. The test material consisted of corn pollen from corn plants, line MON 858. The Cry3Bb1 content was estimated to be 18.8 µg/g fresh weight pollen. The study is procedurally sound and no treatment mortality or behavior change was reported between the dosed and control replicates for the 48-hr exposure period.

The October 2000 and August 2002 SAP reports recommended that non-target testing be focused on species exposed to the crop being registered. The Agency has determined that the non-target organisms most likely to be exposed to the protein in transgenic corn fields were beneficial insects feeding on corn pollen and nectar, and soil invertebrates, particularly Coleoptera spp. Therefore, testing of aquatic invertebrates was performed primarily to satisfy the testing requirements for microbial toxins published in 40 CFR Part 158. No substantial aquatic exposure to Cry3Bb1 protein contained within corn plant tissue is expected except for possibly small amounts of pollen. Several public comments have raised questions about using corn pollen in aquatic invertebrate testing with *Daphnia magna* because corn pollen is thought to be too large for ingestion by these filter feeders. However, there is some observational evidence that daphnids do ingest pollen. As indicated in some study reports reviewed by the Agency, daphnids were actually yellow in color, which can be indicative of ingestion of the yellow pollen test material. However, there is no clear evidence that *Daphnia magna* is capable of ingesting particles as large as pollen. Therefore only a statement of no effect from exposure to pollen, and no statement on lack of toxicity can be made from this study. However, since the Cry3Bb1 protein is confined to corn tissue, and the worst case aquatic EEC is calculated to be 3.9 ng Cry3Bb1 protein/mL, there is no substantial exposure to aquatic invertebrates, and therefore no hazard from the registered used of Cry3Bb1 containing corn is anticipated. As a result, no further aquatic invertebrate testing is required at this time.

iii. Estuarine and Marine Animals

The Estuarine and Marine animal studies are waived for this product because of very low to no potential for exposure to CryBb1 protein from field corn.

iv. Terrestrial and Aquatic Plants

Since the active ingredient in this product is an insect toxin (Bt endotoxin) that has never shown any toxicity to plants, these studies have been waived for this product. Outcrossing issues are addressed below.

3. Terrestrial Invertebrate Testing and Hazard Assessment

Background:

The October 2000 SAP concluded that invertebrates such as earthworms and springtails (collembola) are appropriate indicator species for Cry protein testing because of the specific nature of the Cry protein toxicity to select target species. When it initially reviewed the applications for PIP products that were registered in 1995, EPA considered requiring studies evaluating effects upon the representative beneficial soil invertebrates Collembola and earthworms. The Agency was concerned (1) that such soil organisms may be subject to long-term exposure as a result of soil incorporation of crop residues or when crop residues are left on the soil surface and (2) that adverse effects on such soil organisms could result in an accumulation of plant detritus in fields. Recent reports of exudation of Cry proteins by corn roots throughout the growing season add to this concern. However, the Agency understands that routine agronomic practices have included the long term use of chemical insecticides, which have adverse effects on soil organisms, but there has not been an accumulation of significant amounts of plant detritus in soils. Thus, Cry3Bb1 corn, which is expected to have less impact on these species than chemical pesticides, should not result in any increased build up of plant detritus or Cry proteins at toxic levels. Supporting this conclusion are data received by EPA that indicate that such proteins are known to degrade rapidly in field soils. Cry proteins that are bound to soil particles have been shown to be rapidly degraded by soil microbes upon elution from the soil particles. Therefore, the potential for significant soil buildup and adverse effects to non-target soil organisms are not anticipated. It has been confirmed in published literature that Bt Cry protein released from root exudates and biomass of Bt corn plants has no apparent effect on earthworms, nematodes, protozoa, algae, bacteria, actinomyces and fungi in soil in spite of the fact that enough detectable Cry protein is bound to soil particles to show toxicity to the target pest. These results suggest that despite its presence in soil, the Cry protein released in root exudates of Bt corn, or from the degradation of the biomass of Bt corn, is not toxic to a variety of organisms in the soil environment. It has also been reported that the same degree of Bt Cry protein persistence takes place in soils that have been exposed to repeated Bt microbial spray applications. In addition, new plants grown in Bt containing soil do not take up the Bt protein. Nevertheless, data on insects closely related to the target pest, as well as other studies to address the published data requirements for registration of microbial toxins (40 CFR §158) have been received and reviewed.

a. Single Species Laboratory Testing

i. Effects on Honey Bee Larvae

An acceptable study (MRID No. 449043-10) was conducted based on OPPTS Series 885-4380, Honey bee testing Tier I. This study was conducted in accordance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 160 with certain exceptions that did not affect the integrity of the test.

Testing was conducted with *Bacillus thuringiensis* Cry 3B2.11231 protein (purity 96%; 1.79 mg active protein/mL water; current nomenclature refers to this protein as Cry3Bb1) inoculated directly into larval brood cells prior to capping. Within 18 days after treatments were administered, all larvae emerged from capped brood cells. All of the larvae (100%) treated with Cry3Bb1 protein survived to pupation or "capping"; whereas, 97.5% (2.5% mortality) of the honey bee larvae in the control group survived to pupation. There was no statistical difference ($p=0.05$) in total percent mortality during the larval development or adult emergence stages between treated and control groups. Based on the results presented in the study, it can be concluded that honey bee development and survival are not affected by exposure to the Cry3Bb1 protein. There was 88.8% mortality of larvae treated with the reference substance potassium arsenate which indicated that bees were exposed to the treatments. The LC_{50} for honey bee larvae was determined to be >1,790 ppm Cry3Bb1 protein.

According to the OPPTS Harmonized Testing Guidelines, non-target insects should be tested at 10-100X the field dosage. This test was conducted at an acceptable level 100X the concentration in pollen or 1,790 ppm Cry3Bb1 protein. Since potential exposure of honey bees to Cry3Bb1 will be from pollen, this test was conducted at an appropriate maximum hazard dose. Therefore, no hazard to honey bee larvae and their development is expected from exposure to the Cry3Bb1 protein in corn pollen.

ii. Adult Honey Bee Testing

This study (MRID No. 449043-11) was conducted in accordance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 160 with certain exceptions that did not affect the integrity of the test. This study was conducted based on OPPTS Series 885-4380, Honey bee testing Tier I.

The testing consisted of a control group fed 30% sucrose in deionized water, a reference group fed 100µg/mL potassium arsenate, and a test group fed 360 µg/mL of Cry3Bb1 protein and a water only group. The study concluded that 360 µg/mL Cry3Bb1 protein did not affect survival or behavior of adult honey bees. The maximum hazard dose LC_{50} of >360 µg/mL is 20X the concentration found in pollen. Therefore, no hazard to adult honey bees is expected from exposure to the Cry3Bb1 protein in corn pollen.

iii. Parasitic Hymenoptera Testing

This study (MRID No. 449043-13) was conducted in accordance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 160 with certain exceptions that did not affect the integrity of the test. This study was conducted based on OPPTS Series 885-4340 Non-target Insect Testing, Tier I.

A dietary toxicity study with the parasitic Hymenoptera (*Nasonia vitripennis*) was conducted with *Bacillus thuringiensis* Cry 3B2.11231 (Cry3Bb1) protein (purity 96%; 34.5 mg active protein/mL water). Wasps were tested at rates of 400 and 8,000 ppm Cry3Bb1 protein which is approximately equivalent to 1X and 20X the maximum protein concentration in plant tissue. The LC_{50} for parasitic Hymenoptera was determined to be >8,000 ppm Cry3Bb1 protein. When an adjustment for mortality in the control group is considered, mortality in the 8,000 ppm treatment group is 45%. Although differences in mortality between the control and treatment groups were not significantly different ($p>0.05$), a treatment effect at 20X EECcould not be precluded in this study. At test termination mortality for the 100 ppm potassium arsenate reference group was 33% (24 of 73) and 100% mortality (70 of 70) in the 1,000 ppm reference group.

Based on this test, the LC_{50} for adult parasitic Hymenoptera exposed to dietary Cry3Bb1 is >8,000 ppm. The hazard assessment is based on 4000 ppm Cry3Bb1 protein which is 10X the field concentration in plants. However, because parasitic Hymenoptera do not feed directly on corn plant tissues, minimal exposure of parasitic Hymenoptera to Cry3Bb1 protein is expected. As a result, no hazard to *Nasonia vitripennis* is expected from exposure to MON 863 Cry3Bb1 corn.

The preliminary review of the *Nasonia vitripennis* study was initially found acceptable by the Agency (memorandum from Robyn Rose to Mike Mendelsohn dated May 20, 2002). However the August 27, 2002 SAP concluded that the parasitic Hymenoptera (*Nasonia vitripennis*) testing was not appropriate. The SAP concluded that "[t]he levels of exposure of ... *Nasonia* to active protein were not, for example, determined throughout their respective tests. The test protein ... within a diet broth ... could have degraded considerably." Not only were the procedures in this study questioned by the SAP, the appropriateness of testing this organism is questionable. *N. vitripennis* is a dipteran parasitoid that does not occur in corn fields. A more appropriate parasitoid that occurs in corn fields (*e.g. Tricogramma* or *Macrocentrus grandii*) should be considered. Since *Tricogramma* and *Macrocentrus* are lepidopteran parasitoids, testing another beneficial organism rather than a parasitoid is appropriate. Therefore, the Agency recommends additional maximum hazard dose laboratory testing of a beneficial coleopteran such as a carabid (ground beetle).

iv. Green Lacewing Larva Testing

An acceptable study (MRID No. 449043-12) was conducted in accordance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 160 with certain exceptions that did not affect the integrity of the test. This study was conducted based on OPPTS Series 885-4340 Non-target Insect Testing, Tier I.

Green lacewing larvae were fed the Cry 3Bb1 protein in a moth egg (*Sitotroga* sp.) and water meal diet at rates of 400 and 8,000 ppm which is approximately equivalent to 1X and 20X the maximum protein concentration in plant tissue. There was 20% mortality in the negative control group on day 10. Compared to the negative control, at day 10, there was no significant increase in green lacewing larval mortality when fed 1X (400 ppm) and 20X (8,000 ppm) the maximum Cry3Bb1 protein concentration found in plant tissue. At test termination mortality for the 1,000 ppm reference group was 43% (13 of 30) and 100% mortality in the 10,000 ppm reference group (potassium arsenate). The data show that the LD_{50} for green lacewing larvae exposed to Cry3Bb1 in diet is >8,000 ppm. Based on these results it is not expected that the green lacewing will be adversely affected when exposed to Cry3Bb1 in the field.

The preliminary review of the green lacewing larva study was initially found acceptable by the Agency. However the August 27, 2002 SAP concluded that the green lacewing (*Chrysoperla carnea*) testing was not appropriate. Several public comments also addressed this issue. The SAP concluded that "[t]he levels of exposure of *Chrysoperla* to active protein were not, for example, determined throughout their respective tests. The test protein was held for a week within a diet broth in the *Chrysoperla* test chamber, and could have degraded considerably."

Additional problems were recognized with the *Chrysoperla* laboratory study. Green lacewing are difficult to test in the laboratory because of a high rate of mortality. In this instance (MRID No. 449043-12), the test was terminated after 10 days because there was >20% mortality in the positive control. In addition, it is questionable whether the green lacewings are ingesting the Cry3Bb1 protein that is coated around moth eggs in a diet. Since green lacewing have piercing-sucking mouthparts, they may not be exposed to the protein on the external surface of the egg diet. Therefore, Monsanto should conduct a laboratory insect toxicity test on an alternate organism. The minute pirate bug (*Orius insidiosus*) would be a more appropriate species to test than the green lacewing. *Orius* typically occur in corn fields as egg predators and they typically feed on pollen. Therefore, a laboratory study should be conducted feeding *O. insidiosus* both pollen and purified protein in diet. Feeding *O. insidiosus* Cry3Bb1 protein in diet will allow for a test at the maximum hazard dose; whereas, feeding *O. insidiosus* pollen expressing the Cry3Bb1 protein will provide an evaluation of potential effects from actual exposure scenarios.

v. Lady Beetle Testing

Since the Cry3Bb1 protein specifically targets coleopteran (beetle) insects, particular attention is warranted regarding potential effects of MON 863 on lady beetles. In a memorandum from Robyn Rose to Mike Mendelsohn dated March 10, 2000, in addition to a dietary exposure to the purified Cry protein (44903-14), the Agency requested a test demonstrating the effect of lady beetles feeding on corn pollen containing Cry3Bb1. Monsanto conducted three additional laboratory studies (MRID Nos 453613-01, 453613-02 and 455382-04) on two different lady beetle species (*Coleomegilla maculata* and *Hippodamia convergens*) in response to this request.

Adult Lady Beetle Protein Dietary Study

A diet containing purified Cry protein and honey was fed to the adult lady beetle (*H. convergens)* at rates one and 20 times the maximum protein concentration found in corn leaf tissue (MRID No. 449043-14). When the negative control group reached 20% mortality on day 10 , the results showed no significant differences in the mortality rate between lady beetles fed 400 and 8,000 µg Cry3Bb1/mL of diet. Results from this study showed that the LC_{50} for Cry3Bb1 when incorporated in diet and fed to *H. convergens* is >8,000 µg Cry3Bb1 protein/mL diet. Mortality for the 1,000 and 10,000 µg potassium arsenate/mL diet groups were 55% and 95% respectively at day 10. This demonstrates that toxicity can be measured by mixing a test substance in the lady beetle diet. Lady beetles do not feed on corn plant tissue. They do, however, feed on corn pollen and prey on pest insects that may feed on corn tissue and contain Cry3Bb1 in their gut, thus exposing lady beetles to the Bt protein. There is approximately 390 µg Cry3Bb1/g fresh weight corn tissue. Lady beetle exposure is expected to be significantly lower than this since the corn tissue would be metabolized, eliminated, or otherwise degraded within the prey species. Since the maximum hazard dose LC_{50} was found to be 8,000 µg Cry3Bb1/mL diet which is 20 times higher than maximum expected exposure levels, no hazard from Cry3Bb1 in corn plants to adult lady beetles is anticipated.

Larval Lady Beetle Pollen Feeding Study

At certain times corn pollen may comprise up to 50% of lady beetle larvae's diet. Therefore the effects of corn pollen containing event MON 863 Cry3Bb1 protein on lady beetle larvae (*Coleomegilla maculata*) was evaluated (MRID No. 455382-04). Pollen was fed to lady beetle larvae in a diet consisting of equal amounts of lyophilized tephritid fruit fly eggs and bee pollen. Diets contained 50% pollen (93 µg Cry3Bb1/g fresh pollen weight) since this is the potential level of field exposure and an equal amount of the tephritid fruit fly diet. First instar lady beetle larvae were individually placed in test arenas to avoid cannibalism. There was not a statistically significant difference between developmental time of larvae to pupae and/or adults; nor was there a difference in adult weight survival between larvae fed bee pollen or corn pollen nor was there a difference between larvae fed Bt and non-Bt pollen. There was a significant difference between the reference group (potassium arsenate) and other test groups since no larvae survived in the reference group. The 100% mortality observed in the reference group verified that the lady beetles were ingesting the diet. This test was conducted with pollen levels greater than or equal to levels lady beetle larvae are expected to be exposed to in the field. Therefore, the LD_{50} for Cry3Bb1 expressed in corn pollen is >93 µg/g fresh pollen weight. This study demonstrates that lady beetle larvae will not be adversely affected by Cry3Bb1 field corn.

Adult Coleomegilla maculata* and *Hippodamia convergens Pollen Feeding Studies

Coleomegilla maculata lady beetle adults were fed diets of transgenic corn pollen mixed with fruit fly eggs to determine the potential effects of transgenic pollen to beetles (MRID No. 453613-01). The corn (MON 863) test pollen (assayed at the time of testing) contained the Cry3Bb1 protein at a concentration of 37.4 µg/g pollen. After 30 days of diet exposure, 83.3 and 80.0% of adult *C. maculata* survived in the test and control pollen groups, respectively. While these survival rates were significantly less than that in the assay control group (bee pollen which exhibited 100% survival), there were no significant differences between the test and control pollen groups. All adults in the positive control (arsenate treated corn pollen) died in less than 8 days. Results indicated that transgenic Bt corn pollen expressing the variant Cry3Bb1 protein have no significant negative effects on the survival of *Coleomegilla maculata* adults.

Hippodamia convergens adults were fed diets of transgenic corn pollen in honey to determine the potential effects of transgenic pollen to non-target beetles (MRID No. 453613-02). The corn (MON 863) test pollen (assayed at the time of testing) contained the Cry3Bb1 protein at a concentration of 37.4 µg/g pollen. After 15 days of diet exposure, 84% and 81% of adult *Hippodamia convergens* survived in the test pollen and control pollen groups, respectively. There were no significant differences in survival among the test pollen, control pollen and the assay control (honey only) treatment groups. Only 5% of beetles exposed to the positive control (arsenate treated corn pollen) survived. Results demonstrate that transgenic Bt corn pollen expressing the variant Cry3Bb1 protein had no significant negative effects on the survival of *Hippodamia convergens* adults from dietary exposure.

No adverse effects were detected when *Coleomegilla maculata* and *Hippodamia convergens* were fed MON 863 pollen in diet in the laboratory. Pollen levels fed on by the lady beetles in this study exceeded concentrations that are expected to be encountered in the field. Therefore, it can be concluded the MON 863 will not pose a hazard to lady beetle adults in the field.

vi. Collembola Feeding Study

This study (MRID No. 449043-17) was conducted in accordance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 160 with certain exceptions that did not affect the integrity of the test. This study was conducted based on OPPTS Series 885-4340 Non-target Insect Testing, Tier I

Collembola (*Folsomia candida*) were fed diets consisting of transgenic corn leaf tissue containing Cry3Bb1 protein mixed with dry granulated Brewer's yeast. Diets contained a ratio of 0.50, 5.0 and 50% corn leaf tissue in Brewer's yeast which was equivalent to 8.73, 87.3 and 872.5 µg Cry protein/gram diet respectively. The corn leaf tissue contained 1,745 µg Cry3Bb1 protein/g dried leaf tissue.

These results show a LD_{50} > 872.5 µg/g diet of Cry3Bb1 protein. The study also noted that a diet containing 50% corn leaf tissue expressing the Cry3Bb1 Bt protein (a maximum hazard dose) did not adversely affect reproduction of Collembola. This test was conducted at concentration levels much greater than Collembola are expected to be exposed to in the field. The primary route Collembola would be exposed to Cry3Bb1 in the field is from decaying root tissue (and possibly from pollen to a much lesser degree). MON 863 is expressed in corn roots in the range of 3-66 µg/g which is significantly lower than the levels used in this test.

This study adequately addresses potential concerns for Cry3Bb1 protein expressed in transgenic corn to Collembola (*Folsomia candida*) a representative of beneficial soil insect species. The results of this study demonstrate that Cry3Bb1 proteins found in transgenic corn pose no hazard to soil inhabiting Collembola species, and by inference to other beneficial non-coleopteran soil insects. It is notable that recent recommendations by the October 2000 Scientific Advisory Panel are that invertebrates of different orders than those known to be affected by the Cry protein in question not be tested.

vii. Earthworm Toxicity Testing

Background:

Earthworm feeding studies submitted to the Agency for all of the registered Cry proteins demonstrate that the Cry proteins are not toxic to earthworms at the worst case environmental concentration. Some public comments have voiced concerns as to whether the earthworms actually ingested the Bt Cry proteins when these are incorporated into the soil in the test systems used. Recently published data show that the earthworms do, however, ingest the Bt Cry proteins with the soil without harmful effects. The data also show that there were no significant differences in the percent mortality and weight of earthworms after 40 days in soil planted with Bt or non-Bt corn, in fallow fields, or after 45 days in soil amended with biomass of Bt or non-Bt corn or not amended. The Bt Cry protein was shown to be present in both the casts and guts of the worms.

Cry3Bb1 Earthworm testing:

MRID No. 449043-16

The study was conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Parts 160 and 792; Organization for Economic Development (OECD) Principles of Good Laboratory Practice; and Japan Ministries of Agricultural Forestry and Fisheries (MAFF), with certain exceptions that did not affect the integrity of the test. The testing was conducted based on OPPTS Series 850.6200 Earthworm Subchronic Toxicity Test and OECD Guideline 207. This study meets current testing requirements for assessing risks to earthworms from plant-incorporated protectants derived from *Bacillus thuringiensis*.

The 14-day LC_{50} for earthworms exposed to Cry3Bb1 protein 11231 in an artificial soil substrate was determined to be greater than 570 mg Cry3Bb1 protein/kg dry soil. However, the percent mortality reported was 38%. The mortality in the 57.0 mg/kg group was 8%. It was noted in the study design that the levels of buffer salt in the test groups were higher than expected because of a miscalculation. The actual concentration of sodium bicarbonate salt in the 57.0 and 570 mg Cry3Bb1 protein/kg treatment groups was 70 and 699 mg/kg, respectively. The higher concentrations did not appear to have any influence on the overall conclusions of the study. However, another earthworm study (MRID No. 457571-01) was performed.

MRID No. 457571-01
The submitted study is classified as acceptable, it is scientifically sound, and is consistent with current testing requirements for earthworm hazard assessment. The 14-day LC_{50} for earthworms exposed to purified 11098 Cry3Bb1 (*E. coli* -produced) protein in an artificial soil substrate was determined to be greater than 166.6 mg /kg dry soil (the highest concentration tested), or greater than 20 times the worst case EEC in a corn field. There was no apparent effect of the phosphate buffer on the earthworms. There were no earthworm mortalities in the any of the controls or Cry-protein treated soils during the 14 day study. Changes in average body weights were not statistically different (p>0.05) among the controls and protein-amended soils. There were no other remarkable observations. At the end of the study, mortality in the 10 and 20 mg chloroacetamide/kg soil was 2.5% (1 of 40) and 85% (34 of 40), respectively. Percent mortality of earthworms in the reference substance (chloroacetamide) groups was consistent with historical results, and further confirmed the adequacy and consistency of the protocol used in the definitive test.

The reviewed data show that no adverse effects to earthworms are expected in fields growing Cry3Bb1 corn plants.

viii. Monarch Butterfly Larval Pollen Feeding Study

This study (MRID No. 455382-05) was not required nor requested for Cry3Bb1 because Cry3Bb1 it is a coleopteran active protein that is not expected to affect lepidopterans such as the monarch butterfly. In addition, extensive research conducted on the potential affects of monarch feeding on lepidopteran-active Bt corn pollen has shown a lack of concern for subchronic toxicity. However, due to recent public concern for possible adverse effects of Cry3Bb1 corn on monarchs, Monsanto sponsored this study and submitted it to the Agency for review. This study has demonstrated that corn pollen expressing the Cry3Bb1 protein will not result in subchronic toxic or developmental effects to monarch larvae. Neonate monarch survival was not affected after feeding on milkweed dusted with up to 3200 pollen grains/cm2 expressing Cry3Bb1 for 2, 4 or 10 days of pollen exposure. Larval development, weight gain and milkweed leaf consumption were also not affected by feeding on Bt pollen 96 hours and 10 days after exposure. Pollen densities in the field are not expected to be as great as 3200 grains/cm^2. Pollen densities in the field average 150 grains/cm^2. Levels of 400 and 800 pollen grains/cm^2 would probably be rare. Therefore, results of this study indicate that young monarch larvae (at the most sensitive stage) will not be adversely affected by exposure to corn pollen expressing Cry3Bb1 in the field. However, since Cry3Bb1 expressed in MON 863 is a coleopteran-active protein, the August 27, 2002 SAP concluded that the monarch butterfly was not an appropriate indicator organism to be tested. The SAP recommended testing *Tetraopes* (red milkweed beetles) as a more logical choice than the monarch butterfly.

ix. Insecticidal Activity Spectrum Study

Insecticidal spectrum of activity bioassays were conducted on six Coleoptera Families and two Lepidoptera species (MRID No. 455328-07). A series of six to eight concentrations of CryBb1 protein (1ppm - 8000ppm) in standard insect diet preparations were used to conduct seven day mortality testing. Significant insecticidal activity was seen only in the family Chrysomelidae (corn rootworm and Colorado potato beetle) of the Order Coleoptera. No activity was seen against the cowpea weevil (fam. Bruchidae), lady bird beetle (Coccinellidae), red flour beetle (Tenebrionidae), cotton boll weevil, pepper weevil and rice weevil (Curculionidae). The Lepidoptera corn earworm (Noctuidae) and European corn borer (Crambidae) were also not affected. This efficacy study was reviewed for environmental assessment purposes to expand the number of insect species examined for possible toxicity of the Cry3Bb1 protein. The results confirm the assertion that Bt Cry proteins have a very specific and narrow range of target species.

b. Field Evaluation of Cry3Bb1 Corn Exposure on Non-target Invertebrates

i. Introduction

The Scientific Advisory Panels (October, 2000 and August, 2002) recommended that non-target testing be focused on species exposed to the crop being registered. The Agency has determined that the non-target organisms most likely to be exposed to the Cry3Bb1 protein in transgenic corn fields were beneficial insects feeding on corn pollen and nectar, and soil invertebrates, particularly Coleoptera spp. In addition to extensive and difficult maximum hazard dose single species soil coleopteran toxicity testing followed by an extrapolation from the results to a community risk assessment, and the fact that all of the species cannot be tested in the laboratory, direct field test and field census data on coleopteran insect effects and abundance were requested, received and evaluated. These studies are being conducted in several States by Monsanto Co. and several independent University scientists. The results of these studies are summarized below. Some of the submissions consists of preliminary results of studies in progress. The Agency is requesting that Monsanto submit the final reports of these studies as they become available.

These preliminary field and field census data with the study design methodologies have been presented to a Scientific Advisory Panel (August, 2002). The SAP commented that the study designs lack appropriate statistical power, but that methodology for conducting statistically valid field census studies at the scale necessary to determine ecosystem effects is not available. Such methodology is yet to be developed. As a result, the Agency is reviewing the available field studies as data supplemental to the maximum hazard dose single species laboratory testing but useful for short range assessment of non-target invertebrate abundance in Cry3Bb1 corn test plots. It is an accepted practice in the Office of Pesticide Programs to use the trends seen in several supplemental studies for hazard assessment when a perfect study is not available.

ii. Preliminary Invertebrate Field Census data (MRID No. 455382-06)

These two year Tier IV field studies are intended to supplement the Tier I maximum hazard dose findings. Invertebrates in Cry3Bb1 corn field plots were sampled from the soil, soil surface and foliage. Soil-dwelling invertebrates were collected using a "pan trap" which utilized a modified Burlese extraction method. Surface-dwelling invertebrates were sampled in the field with pitfall traps. Foliage-dwelling invertebrates were monitored by yellow sticky traps (Pherocon AM™) set in the field at canopy level and adjusted as the season progressed. Sampling for lady beetles was also done using a drop cloth technique. Preliminary results do not show any MON 863 corn related adverse effect on non-target and beneficial invertebrate abundance in the field. The final report (MRID No. 457916-01) is reviewed below.

iii. Final Invertebrate Field Census data (MRID No. 457916-01)

Methods:

During the 2000 and 2001 growing seasons, event MON 863 CRW-protected corn and non-transgenic corn (hybrid RX670) were grown in Warren County near Monmouth, IL. Corn was planted in both fields the previous year and soybeans were planted two years prior to conducting these field trials. All experimental plots were managed according to typical cultural practices of commercially grown corn in the region and included the application of the herbicides acetochlor and atrazine after planting and before emergence.

During the 2000 and 2001 growing seasons, Bt (MON 863) and control hybrids (RX670) were the main plots planted in a split-plot design with four replications planted 20 ft apart. Rows were planted 30 inches apart, seeded at a rate of approximately 1.7 seeds/ft and planted 1.5 - 1.75 inches deep. Plots (240 ft × 60 ft) were divided into 24 row subplots (60 ft × 60 ft) that served as replications receiving one of 4 insecticide regimes. Insecticide treatments of the Bt and non-Bt plots included: 1. No insecticide; 2. Seed treated with Gaucho® prior to planting; 3. The granular insecticide Force 3G® applied and incorporated in furrows at planting; and 4. A foliar insecticide, Pounce 3.2 EC®, applied at the V10 and R2 corn growth stages to control 1st and 2nd generation CRW adults. A 4-row buffer of non-transgenic corn was planted around each plot to minimize edge effects from adjacent subplots.

Data were collected on agronomic and phenotypic characteristics, pest (insect and disease) susceptibility, soil quality and fertility, microbial populations and non-target invertebrate abundance. Eight inch deep soil samples were taken to evaluate quality and fertility. Four samples were taken during the growing season and two samples post-harvest in 2000; two samples were taken during and after the growing season in 2001 for a total of ten samples. Microbial populations were evaluated from test and control plots that received no insecticide regime a total of 14 times during the growing season from the top six inches of soil within six inches of the rhizosphere. Samples were taken in 2000 and 2001 during the V2, V4, V8, R1 and R6 stages as well as after tillage and the following spring. Soil samples from the 2000 growing season were analyzed for bacteria, mold and yeast by a heterotrophic plate count method. Four of the seven soil samples collected during the 2001 growing season were analyzed for bacteria, actinomycetes and fungi by the "viable plate count method."

Soil-dwelling invertebrates were collected from root balls including soil during the V6, V10 and R1 growth stages during the 2000 and 2001 growing seasons. Three eight inch root balls were collected during the V6, V10 and R1 corn growth stages from all control and test plots and processed through a Berlese funnel to extract invertebrates. Earthworms were also collected from soil samples by hand sorting. Ground surface-dwelling invertebrate were collected from all test and control plots under all insecticide regimes using pitfall traps. Four pitfall traps were placed in each subplot from the V6 to R4 corn growth stages for three-day periods. Key invertebrates from pitfall traps were counted and identified to family level. Flying and foliage-dwelling invertebrate were collected from each subplot using yellow sticky traps. Three traps per subplot were placed at canopy level from the V6 to R4 corn growth stages. Traps were left in the field for seven days and all key taxa were counted and identified to family or genus level. Data were analyzed using a mixed linear repeated measures model for each invertebrate collected by each sample method.

Results:

Among the three sample methods (soil, pitfall and sticky trap) there was a total of 156,572 organisms from 16 orders and 36 families identified during the 2000 and 2001 growing seasons. Collected invertebrates included pests, predators, parasitoids, detritivores and decomposers. The predominant non-target invertebrates collected in each sample method are summarized in Table 2.

Table 2. Predominant non-target invertebrates collected in each sample method

Sample Method	**Order (Family)**
Soil and Root Samples (soil-dwelling invertebrate)	Diplura (Japygidae), Chilopoda, Aranea, Acari, Oligocaeta (earthworms), Coleoptera (Carabidae, Staphylinidae, Nitidulidae, Lanthridiidae), Hymenoptera (Formicidae)
Pitfall Trap Samples (ground surface-dwelling invertebrate)	Orthoptera (Gryllidae), Coleoptera (Carabidae, Staphylinidae, Nitidulidae, Scarabeidae, Chrysomelidae), Hymenoptera (Formicidae), Araneae, Chilopoda
Yellow Sticky Trap Samples (flying & foliage-dwelling invertebrate)	Coleoptera (Chrysomelidae, Nitidulidae, Coccinellidae), Hymenoptera, Homoptera (Aphididae, Cicadellidae), Hemiptera (Anthocoridae), Diptera (Syrphidae), Neuroptera (Chrysopidae, Hemerobiidae), Aranea

Overall, there was no statistical difference between MON 863 and non-Bt plots (RX670) in the abundance of the predominant invertebrates collected in soil samples. According to the soil sample data, the number of Japygidae (diplurans) did not differ between Bt and non-Bt plots; however, there were significantly less collected in the insecticide treated plots. Of the coleopteran insects collected, there were generally more carabids (ground beetles) and staphylinids (rove beetles) than nitidulids (sap beetles) and lanthridiids (minute brown scavenger beetles). There was no statistical difference in the number of coleopteran insects captured between the MON 863 and non-transgenic corn isolines. Statistical analysis showed that insecticide treatments significantly reduced the number of carabids in 2000 and the number of staphylinids on the last sample date in 2001. Invertebrates from the Araneae (spider) and Acari (mite) families were also not significantly different between Bt and non-Bt plots; however the number of acarids (mites) were statistically greater in the plots treated with foliar insecticides in 2001. The number of chilopods (centipedes), the most abundant non-insect arthropod sampled, was not different between Bt and non-Bt isolines, but more were collected in plots treated with seed, soil and foliar insecticides in 2001 particularly the MON 863 treated plots. Although the number of earthworms collected did not differ between Bt and non-Bt plots, there were significantly less earthworms in the plots treated with foliar insecticides than the other insecticide regimes. The hand-sorting method also showed no differences in the number of earthworms collected in MON 863 and non-transgenic plots, nor was there a difference found between insecticide regimes.

Overall, there was no statistical difference between MON 863 and non-Bt plots (RX670) in the abundance of the predominant invertebrates collected in pitfall trap samples. Of the coleopterans captured in pitfall traps, there were more nitidulids (sap beetles), carabids (ground beetles) and staphylinids (rove beetles) captured than chrysomelids and scarabids collected. There was no statistical difference between MON 863 and RX670 field-plots in the overall number of coleopterans collected in pitfall traps. The different insecticide regimes tested resulted in varied and inconsistent effects on the abundance of the predominant Coleoptera sampled. The number of gryllids (crickets) and formicids (ants) collected in pitfall traps was not statically different between Bt and non-Bt plots or different insecticide regimes. Chilopod (centipede) and Araneae (spider) abundance was not different between MON 863 and RX670 plots nor was there an affect from insecticide regimes in most cases. Statistical analysis showed that there were significantly fewer Araneae (spider) in the plots with soil and foliar insecticide treatments in 2000.

Based on yellow sticky trap counts, there were consistently less corn rootworm (CRW; *Diabrotica virgifera* and *Diabrotica barberi*) in the MON 863 plots than the non-Bt plots in all insecticide regimes. However, the difference in the number of CRW captured was not statistically different. In both 2000 and 2001, there were no significant differences between MON 863 and RX670 plots across all insecticide regimes in the number of nitidulids (sap beetles), coccinellids (lady beetles), aphidids (grass hoppers), cicadellids (potato leaf hoppers), braconids (*Macrocentrus grandii* - a parasitoid), syrphids (syrphid or hover flies), hemerobiids (brown lacewing), chrysopids (green lacewing) and Araneae (spiders). There was a reduction in the abundance of *Coleomegilla maculata* (lady beetles) and *M grandii* and an increase in *Empoasca fabae* (potato leaf hopper) in plots receiving a foliar spray. Syrphid fly abundance was reduced in 2000 by soil insecticide treatments.

Field Census Summary:

Data collected during the 2000 and 2001 growing seasons indicate that MON 863 and RX670 corn are agronomically and phenotypically equivalent and there are not differences in their susceptibility to pathogens. Soil quality and fertility were also found to be consistent among MON 863 and RX670 field plots. Sampling data collected in 2000 and 2001 also showed that there are no overall differences in the abundance of non-target invertebrate collected in MON 863 and RX670 plots. However, corn pests such as *D. virgifera*, *C. pulicaria* and *R. maidis* as well as predators (e.g., *O. insidiosus* and *C. maculata* [lady beetle], parasitoids (e.g., *M. grandii*) and decomposers (e.g., earthworms and diplurans) were significantly impacted by insecticide regimes. Foliar sprays and soil treatments resulted in the greatest impact on non-target organisms such as carabids, spiders, *O. insidiosus* (a generalist predator), *C. maculata* (lady beetle) and *M. grandii* (parasitic wasp). Therefore, the report concluded: "MON 863 had less impact on certain beneficial insects compared to traditional insecticide control programs, especially soil and foliar applications. Thus, the use of MON 863 for corn rootworm control can lead to reduced use of insecticides and increased compatibility with Integrated Pest Management programs in corn."

Field Census Conclusions:

According to the data submitted to the Agency by Monsanto, MON 863 corn does not adversely impact the abundance of non-target invertebrate found in corn fields. However, plot size (240 ft × 60 ft plots divided into 24 row 60 ft × 60 ft subplots) was small and only replicated four times. In addition, each plot only included three root and sticky trap samples and four pitfall trap samples. The August, 2002 SAP concluded that field experiments must be appropriately designed to provide a measure of ecological impacts. In addition, the SAP opinion was that a two year field study would not be sufficient to determine if MON 863 corn will have long term impact on non-target invertebrates. Several public comments also expressed this concern. Short-term field studies are not adequate to draw conclusions on the variations in non-target invertebrate populations. Large field-scale studies conducted for at least three to four years would be needed to draw a conclusion on non-target impacts. The Panel generally concluded that "the state-of-the science" needed for long-term studies must improve for the research to be appropriately conducted to provide meaningful results. The statistical power (avoiding Type II experimental error) needed to gain useful results from field studies would require very large fields, more replications and more samples per plot (e.g., 10 soil and pitfall samples) plus the addition of visual plant samples (e.g., >50/plot). Since the endpoint for field census studies has not been determined, it is difficult to determine how large the fields should be, how many replications are needed and how many samples per plot are needed to achieve appropriate statistical power. Therefore, additional field census studies should not be conducted until the endpoints and logistics of the study have been determined. If Tier I maximum hazard dose single species laboratory studies show a hazard, intermediate field or semi-field studies between laboratory and full-scale field studies should be conducted. Additional full scale field or semi-field studies with appropriate end points and statistical power should also be considered based on recommendations of the August 27, 2002 SAP.

However, the submitted field census data demonstrating an abundant presence and diversity of invertebrates in the corn CryBb1 corn field are useful for short term hazard assessment as supplementary information which shows the same no-hazard trend seen in the maximum hazard dose single species laboratory testing.

Year 2001: Field and Laboratory invertebrate studies (MRID No. 456530-03)

A summary of preliminary findings from several one year supplemental higher Tier field and laboratory studies was submitted (MRID No. 456530-03). These studies were not triggered by Tier I maximum hazard dose testing data; however they appear to have value for assessing possible for long term invertebrate population shift effects. These studies are being conducted in Kansas, Nebraska, Illinois, Virginia, South Dakota and New York to evaluate the ecological impact of MON 863 Bt corn grown under different insecticide regimes on abundance of non-target organisms relative to non-transgenic corn. The submitted summary of preliminary findings shows some possible effects of MON 863 on corn field insects. Preliminary data are being analyzed which suggest an increase in Coccinellid (ladybeetle) larvae, an increase in abundance of mites on MON 863 residues, a laboratory study showing significantly reduced root population and egg production of the plant pathogenic nematode *M. incognita*, a reduction in the population of the non-pathogenic *C. elegans* in root extracts (no effects in soil leachate were seen), and no effects were noted on the entomo-pathogenic nematode *S. carpocapsae*. It was also reported that of 14 species of Collembola, a decrease in one species was noted, although no effect was seen on total Collembola abundance. One inconclusive study found a decrease in Carabids; however these data are suspect because of limitations in movement of ground dwelling insects due to problems with plot layout designs. The studies are being repeated in more adequately rearranged and randomized plots. A final report of these studies must to be submitted to the Agency for review.

4. Soil Degradation Studies (Environmental Fate)

Soil organisms may be exposed to Cry3Bb1 protein by exposure to roots, incorporation of above ground plant tissues into soil after harvest, or by pollen deposited on the soil. Root exposure may occur by feeding on living or dead roots or, theoretically, by ingestion or absorption after secretion of the Cry protein into the soil. In addition, some evidence suggests that Cry proteins while bound to some soil components, e.g. clays and humic acids, are recalcitrant to degradation by soil microorganisms, but without eliminating their insect toxicity. Several factors influence either the affinity of binding or the rate of degradation. In particular, pH near neutrality generally substantially increases degradation. Corn does not grow well below ~pH 5.6 and therefore most corn growing soils are expected to be at a higher pH. Therefore, under most production conditions, corn would not be grown on soils that would inhibit the rate of degradation compared to what is seen at near neutral pH. Nevertheless, these issues are being evaluated on a case-by- case basis by environmental fate studies designed to determine the rate of Cry protein degradation over sufficiently long periods to assure an accurate assessment of degradation in agricultural soils.

MRID No. 451568-04
This study was conducted in accordance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 160.

Methods:

An insect bioassay and an ELISA was conducted to measure the level of functional and non-functional Cry3Bb1 protein present in field soil samples. The amount of lyophilized corn tissue added to the field soil in this study was based on the amount of plant tissue that could potentially be incorporated into the top six inches of soil under field conditions. Since field incorporation of plant tissue usually will not take place until the Fall, this amount of Cry3Bb1 protein represents the worst-case scenario during the growing season, including possible exudation of Cry protein through the roots into soil. Based on this calculation 0.03 g (rounded up from 0.028) dry weight plant tissue was added to each gram of dry sandy loam field soil (from Fayette County of Lexington, KY.); therefore, 3% of the dry weight of soil was dry weight of lyophilized plant tissue. An additional test was conducted with 10% of the soil containing lyophilized dry weight plant tissue (0.10 g leaf tissue/g soil). Insect bioassays included a mixture of test and control substances with an agar-based insect diet added to wells of bioassay trays. Colorado potato beetle (CPB; *Leptinotarsa decemlineata*) larvae were added one larva/well and each treatment bioassay was replicated twice for a total of 16 CPB/replicate. CPB are more sensitive to the Cry3Bb1 protein then CRW and were, therefore, expected to result in a more measurable response than CRW, the target species. In addition to an insect bioassay, an ELISA was conducted to measure the level of Cry3Bb1 protein present in samples. However, the ELISA test will only show extractable protein and does not distinguish between functional and non-functional proteins.

Results:

Results from this study show the DT_{50} and DT_{90} (degradation time) for Cry3Bb1 in leaf tissues protein in sandy loam soil based on ELISA test to be 2.76 and 9.16 days. The 21 day ELISA sample was the last to show traces of Cry protein. At 28 days the Cry3Bb1 protein was below the detection level. However, the value of these results needs to be considered with regard to biological activity because it is unknown if the extractable protein in the ELISA test was functional or non-functional. Therefore the insect bioassays were performed with CPB and determined the DT_{50} and DT_{90} to be 2.37 and 7.87 days respectively. The no-detection level was in the range of the results obtained by ELISA.

Conclusion:

Based on these results Cry3Bb1 protein does degrade rapidly and does not accumulate in sandy loam soil. However, corn is not necessarily grown in sandy loam soil in all regions. Corn is grown in other soil types such as clay loam and silt loam soils in various regions of the U.S. Testing clay soils would, therefore, be considered a "worst case scenario." In addition, this test does not account for all plant tissue such as roots, or root exudation of the Cry protein in the field. It is possible that root tissue is degrading slower then leaf tissue in the soil which may result in a longer duration of degradation time of the Cry3Bb1 protein. Therefore, it was recommended that field testing should be continued in a variety of soil types including clay and humic acid soils, over a three year period of time, to determine the long-term degradation rate and accumulation/persistence of Cry3Bb1 protein in soil.

Since the submission and review of the above study (MRID No. 451568-04), the Agency convened a Scientific Advisory Panel (SAP) on August 27, 2002 to address corn rootworm plant incorporated protectant (PIP) non-target issues including the degradation of the Bt protein in soil. The following is a review of a second aerobic soil degradation study (MRID No. 457571-02) designed to address some of the deficiencies identified in the first study above, and a review of comments made by the August SAP as well as by several public comments.

MRID No. 457571-02

This study is intended to address the issues raised in the study (MRID No. 451568-04) described above. That study was classified as "[s]upplemental to conducting the study in the field with all plant tissue incorporated into the soil in fields that have had MON 863 corn grown for one to three consecutive years. Studies should also be conducted in a variety of soil types particularly soil high in clay and humic acids" as well as other tissue, particularly roots. In addition, the previously submitted aerobic soil degradation study (MRID No. 451568-04) utilized plant tissue from Cry3Bb1 transformation event MON 859 rather than MON 863. The second submission (MRID No. 457571-02) utilized MON 863 corn root and shoot tissue.

Methods:

The test substance consisted of finely ground and lyophilized root and shoot tissue of the corn event MON 863 containing the Cry3Bb1 insecticidal protein. The concentration of Cry3Bb1 protein in the lyophilized corn tissue, determined by ELISA, was 487 µg/g in the root and 468 µg/g in the shoot. A purified Cry3Bb1 protein obtained from a genetically modified *E. coli* strain containing the identical sequence as the MON 863 corn was also included in the study. MON 863 corn shoot and root tissue were collected from field grown plants in Richland, IA. The test matrix consisted of three soils collected from the top six inches, Horizon A, of corn fields in Carlyle, IL, Monmouth, IL and Richland, IA. Soil properties for these three soils (Table 3) and a microbial analysis were characterized. Soil viability was confirmed at test initiation and at approximately four and eight weeks of incubation. Soils were shown to remain active and viable throughout the study (144-228 microbial biomass carbon/50 g soil). The highest concentration of Cry3Bb1 protein in roots 35-days after planting is 66 µg/g. On this basis the maximum field loading of Cry3Bb1 in corn shoots is 3.93 µg/g soil (equivalent to 8 mg shoots/g soil) and for corn root tissue the loading is 2.79 µg/g soil (equivalent to 6 mg of roots/g soil). For additional conservatism, the maximum values were exaggerated 3X for dosing of soils. Therefore, 24 mg of shoot tissue and 18 mg of root tissue was added to soils. Also, the purified Cry3Bb1 control dosing concentration was exaggerated ~25X to model the unlikely scenario that large amounts of protein would be exuded by roots into the soil throughout the growing season. Approximately 48 µg of purified protein was added to 0.5 g of soil. Soils from the three test locations were air dried and dosed with the Cry3Bb1 test substance at these rates. All vials were mixed thoroughly and soil moisture adjusted with deionized water to obtain soil moisture of 75% field capacity at 0.33 bar.

Table 3. Physicochemical characteristics of soils collected from Horizon A (top 6 inches).

Parameter		***Soil Source***		
		Carlyle, IL	**Monmouth, IL**	**Richland, IA**
USDA Textural Class		Silt Loam	Silt Loam	Silt Loam
Particle Size Distribution (%)	Sand Silt Clay	215821	185626	136225
Bulk Density (g/cm^3)		1	1.03	1.07

Parameter		***Soil Source***		
		Carlyle, IL	**Monmouth, IL**	**Richland, IA**
% Organic Matter		2.5	4.6	4
CEC (meq/100g)		16.6	23.9	20.7
Field Moisture Capacity	@ 1/3 Bar @ 15 bar	28.2 15.7	30.7 17.9	30.3 17.2

Table copied from page 29 of 80 of MRID No. 457571-02

Results:

Cry3Bb1 protein levels in soil sample extracts determined by ELISA show that Cry3Bb1 protein concentrations were near or below the ELISA detection limit (LOQ) of 0.16 µg/g after 2 months of incubation. After two months of soil incubation, Cry3Bb1 concentrations were at least an order of magnitude below the initial concentration of Cry3Bb1 in the dosed samples. The DT_{50} values for all dosing regimes and soil types ranged from 0.6 days to 2.3 days and the DT_{90} values ranged from 4.03 days to 50 days (Table 4 reproduced from page 36 of the study submission). Purified Cry3Bb1 protein degraded faster than when corn shoot or root tissue was applied. Visual observation verified that root tissues are slower to degrade in soil than shoot tissue. These results also indicate, as expected, that the longer DT_{50} and DT_{90} values for corn tissues are due to the time required for the tissue to decay and for the Cry3Bb1 protein to move from tissue to soil. In addition, the rapid degradation (DT90 4.0 to 5.2 days) of the purified Cry3Bb1 protein suggest that any Cry3Bb1 protein reaching the soil by root exudation or release from slowly decaying plant tissue would be >90% degraded in less than six days.

Table 4. DT50 and DT90 estimates for the dissipation of Cry3Bb1 protein in soils

Protein Source	**Soil Source**	**DT50**[a] (days)	**DT90**[b] (days)
Purified Protein	Carlyle, IL	**0.63** (0.49, 0.78)[c]	**4.03** (3.67, 4.42)
	Monmouth, IL	**0.64** (0.50,0.80)	**4.18** (3.77, 4.62)
	Richland, IA	**0.73** (0.50, 1.00)	**5.23** (4.48, 6.07
MON 863 Corn Root Tissue	Carlyle, IL	**1.74** (0.78, 3.20)	**27.29** (14.52, 50.58)
	Monmouth, IL	**1.19** (0.86, 1.57)	**12.48** (9.90,15.67)
	Richland, IA	**2.27** (1.68, 2.98)	**50.02** (34.57, 72.18)

Protein Source	**Soil Source**	**DT50**[a] (days)	**DT90**[b] (days)
MON 863 Corn Shoot Tissue	Carlyle, IL	**1.45** (0.82, 2.30)	**18.68** (12.61, 27.46)
	Monmouth, IL	**0.90** (0.61, 1.24)	**7.43** (6.01, 9.12)
	Richland, IA	**1.77** (1.40, 2.21)	**28.59** (23.16, 35.23)

[a]DT50=Time to 50% dissipation of original protein concentration
[b]DT90=Time to 90% dissipation of original protein concentration
[c]Lower and upper 95% confidence interval on the DT value

Conclusions

It is difficult to determine a DT_{50} or DT_{90} for Cry3Bb1 expressed in corn tissue in the field from this study because corn shoot and root tissue were analyzed separately and not all plant material was included. Therefore, methods utilized in this study do not represent actual field conditions. It is unknown whether these laboratory results can be adequately correlated to the field. Additional field studies should be conducted that include the incorporation of all non-harvested plant tissue in a variety of soil types particularly areas high in clay (>26% tested here) and humic acids. These studies should be conducted for at least one growing season after harvest and continue until no Cry3Bb1 protein is detected. In addition the persistence of the Cry3Bb1 protein under less than optimum conditions (e.g., high or low temperatures; high or low soil moisture content) should be examined. Additional studies conducted to address the degradation of Cry3Bb1 protein in the soil should include an insect bioassay utilizing a known sensitive species such as the Colorado potato beetle.

These conclusions are based in part on the August 27, 2002 SAP and several public comments. The Panel concluded that several different soils should be examined and monitored for a minimum of one growing season after harvest and continued until the Cry3Bb1 protein can no longer be detected. The Panel also recommended that an additional sample or two should be examined to verify that an analytical error was not the cause for the lack of detection. According to the Panel, at least two additional soil types should be evaluated for Cry3Bb1 persistence. Soils that are high in organic matter and clay should be concentrated on since there is the highest potential of persistence in these soil types. However, additional soils should also be considered. The Panel also recommended that the soil degradation studies be conducted under less than optimum conditions such as high or low temperatures or high or low moisture content. Since corn roots grow deep into the soil to areas with reduced microbial activity, degradation rates may be reduced. Therefore, degradation of Cry3Bb1 from deep sites should also be examined. The Panel also addressed the protein source that is appropriate for the soil degradation studies. Future studies should utilize plant material that is representative of actual field conditions. For example, whole plant tissue should be incorporated. Plant tissue should not be ground prior to incorporation because it artificially increases the surface area exposed to microorganisms which may lead to an increase in the rate of degradation of the protein. Since more protein may be present than is detected by an ELISA, an insect bioassay using a sensitive species such as the

Colorado potato beetle should be conducted. The SAP concluded that "[r]eal life or true persistence is likely to be equal to or less than that measured with ELISA." If an ELISA is conducted, the results should be compared to results from a beetle bioassay.

This study, although rated as supplemental, provides further evidence that the Cry3Bb1 protein in corn event MON 863 produces no short term risk of unreasonable adverse effects for the environment. Because the Agency believes that additional studies would be useful in completing the database for long term effects assessment, it is requesting additional supplementary studies regarding Cry3Bb1 protein degradation in soil.

5. Effects on Soil Microorganisms

Published studies on the impact of transgenic Cry producing plants indicates that adverse effects on soil microorganisms are unlikely. No effects have been seen due to the protein itself, and only a minimal, transient increase observed in soil microbes attributed to the transgenic cotton plant tissue rather than the Cry protein expressed in that tissue. No adverse effects have been observed in a similar season long field study with Cry3A potato.

6. Horizontal Transfer of Transgenes from Bt Crops to Soil Organisms

The Agency has evaluated the potential for horizontal gene transfer (hgt) from Bt crops and has considered possible risk implications if it occurred. Several experiments published in the scientific literature have been conducted to assess the likelihood of hgt, and have been unable to detect gene transfer under typical conditions. Hgt has only been detected under conditions designed to favor transfer. In addition, the genes that have been engineered into the Bt crops are mostly found in, or have their origin in, soil inhabiting bacteria. Soil is also the habitat of anthrax, tetanus and botulinum toxin producing bacteria. No transfer of these genes or toxin to other microorganisms or plants has been detected. Therefore, the Agency concluded that hgt is at most an artificial event, and the traits engineered into the Bt crops are already present in soil bacteria or are unlikely to have selective value for soil microorganisms. In considering these data the Agency further concludes that there is no significant risk from hgt from the transgenes found in the Cry3Bb1 producing corn.

7. Gene Flow and Weediness Potential

The movement of transgenes from the host plant into weeds has been a significant concern for the Agency due to the possibility of novel exposures to the pesticidal substance. The Agency has determined that there is no significant risk of gene capture and expression of Cry3Bb1 protein by wild or weedy relatives of corn in the U.S., its possessions or territories. In addition, the USDA/APHIS has made this same determination under its statutory authority under the Plant Pest Act.

Under FIFRA, the Agency has reviewed the potential for gene capture and expression of the *B.t.* endotoxins by wild or weedy relatives of corn, cotton, and potatoes in the U.S., its possessions or territories. *B.t.* plant-incorporated protectants that have been registered to date have been expressed in agronomic plant species that, for the most part, do not have a reasonable possibility of passing their traits to wild native plants. Feral species related to these crops, as found within the United States, cannot be pollinated by these crops (corn, potato, and cotton) due to differences in chromosome number, phenology (*i.e.*, periodicity or timing of events within an organism's life cycle as related to climate, *e.g.*, flowering time) and habitat. The only exception, however, is the possibility of gene transfer from *B.t.* cotton to wild or feral cotton relatives in Hawaii, Florida and the Caribbean.

The Scientific Advisory Panel meeting held on October 18-20, 2000 further discussed the matter of gene flow and offered some issues for consideration in this matter. The panel agreed that the potential for gene transfer between corn (maize) and any receptive plants within the U.S., its possessions and territories was of limited probability and nearly risk free.

Concern over the potential for species related to maize (*Zea mays* ssp. *mays*), such as *Tripsacum* species and the teosintes, as potential recipients of gene flow from genetically modified *Zea mays* indicated a need for review of what is known related to gene flow potential of *Zea mays*. Some *Zea* spp., such as the teosintes, are known to be interfertile with maize and are discussed as potential recipients of pollen directed gene flow from maize. This issue is of particular concern based upon the increased planting of genetically modified maize. Therefore, the Agency conducted a reevaluation in early 2000, the results of which are reported here.

a. *Zea mays* ssp. *mays* - Maize - General Biology

Zea mays is a wind-pollinated, monoecious, annual species with imperfect flowers. This means that spatially separate tassels (male flowers) and silks (female flowers) are found on the same plant, a feature that limits inbreeding. A large variety of types are known to exist (*e.g.*, dent, flint, flour, pop, sweet) and have been selected for specific seed characteristics through standard breeding techniques. Maize cultivars and landraces are known to be diploid (2n = 20) and interfertile to a large degree. However, some evidence for genetic incompatibility exists within the species (*e.g.*, popcorn x dent crosses; Mexican maize landraces x Chalco teosinte). *Zea mays* has been domesticated for its current use by selection of key agronomic characters, such as a non-shattering rachis, grain yield and resistance to pests. The origin of corn is thought to be in Mexico or Central America, based largely on archaeological evidence of early cob-like maize in indigenous cultures approximately 7200 years ago.

A recent study has indicated that cross-pollination of commercial maize cultivars at 100 ft downwind from the source of genetically modified maize was 1 % and this proportion declined exponentially to 0.1 % at 130 ft and further declined to 0.03 % at 160 ft. At 1000 ft, the farthest distance measured, no cross-pollination was detected (Jemison and Vayda, 2000). For production of Foundation Seed, a distance of 660 ft has been generally required to mitigate outcrossing between different genotypes. The relatively large size of corn pollen and its short viability period under most conditions reduce long distance transfer for purposes of outcrossing (Schoper, personal communication,1999). Under conditions of high temperature or low humidity, corn pollen may only survive for a matter of minutes. Under more favorable conditions in the field or with controlled handling in the laboratory, pollen life may be extended to several hours.

b. *Tripsacum* species - Gama Grass - General Biology

Close relatives of corn or maize are found in the genus *Tripsacum.* Sixteen species of *Tripsacum* are known worldwide and generally recognized by taxonomists and agrostologists; most of the 16 different *Tripsacum* species recognized are native to Mexico, Central and South America, but three occur within the U.S. In the Manual of Grasses of the United States, A. S. Hitchcock (revisions by Agnes Chase; 1971) reports the presence of three species of *Tripsacum* in the continental United States: *T. dactyloides*, *T. floridanum* and *T. lanceolatum*. Of these, *T. dactyloides*, Eastern Gama Grass, is the only species of widespread occurrence and of any agricultural importance. It is commonly grown as a forage grass and has been the subject of some agronomic improvement (*i.e*., selection and classical breeding). *T. floridanum* is known from southern Florida and *T. lanceolatum* is present in the Mule Mountains of Arizona and possibly southern New Mexico.

For the species occurring in the United States, *T. floridanum* has a diploid chromosome number of $2n = 36$ and is native to Southern Florida; *T. dactyloides* includes $2n = 36$ forms which are native to the central and western U.S., and $2n = 72$ forms which extend along the Eastern seaboard and along the Gulf Coast from Florida to Texas, but which have also been found in IL and KS; these latter forms may represent tetraploids ($x = 9$ or 18; Lambert, personal communication, 1999); and *T. lanceolatum* ($2n = 72$) which occurs in the Southwestern U.S. *Tripsacum* differs from corn in many respects, including chromosome number (*T. dactyloides* $n = 18$; *Zea mays* $n = 10$). Many species of *Tripsacum* can cross with *Zea*, or at least some accessions of each species can cross, but only with difficulty and the resulting hybrids are primarily male and female sterile (Duvick, personal communication, 1999; Galinat, 1988; Wilkes, 1967). *Tripsacum*/maize hybrids have not been observed in the field, but have been accomplished in the laboratory using special techniques under highly controlled conditions.

Eastern Gama Grass is considered by some to be an ancestor of *Zea mays* or cultivated maize (Mangelsdorf, 1947), while others dispute this (Galinat, 1983; Iltis, 1983; Beadle 1980), based largely on the disparity in chromosome number between the two species (maize $n = 10$; Gama Grass $x = 9$ or 18, with diploid, triploid and tetraploid races existing; $2n = 36$ or 72), as well as radically different phenotypic appearance. Albeit with some difficulty, hybrids between the two species have been made (Mangelsdorf and Reeves, 1939; Chet DeWald, personal communication;1999). In most cases these progeny have been sterile or viable only by culturing with *in vitro* 'embryo rescue' techniques.

Even though some *Tripsacum* species occur in areas where maize is cultivated, gene introgression from maize under natural conditions is highly unlikely, if not impossible (Beadle, 1980). Hybrids of *Tripsacum* species with *Zea mays* are difficult to obtain outside of the controlled conditions of laboratory and greenhouse. Seed obtained from such crosses are often sterile or progeny have greatly reduced fertility. Approximately 10 - 20% of maize-*Tripsacum* hybrids will set seed when backcrossed to maize, and none are able to withstand even the mildest winters. The only known case of a naturally occurring *Zea* - *Tripsacum* hybrid is a species native to Guatemala known as *Tripsacum andersonii*. It is 100% male and nearly 99% female sterile and is thought to have arisen from gene flow to teosinte, but the lineage is uncertain (Doebley, personal communication, 2000). *Zea mays* is not known to harbor properties that indicate it has weedy potential and, other than occasional volunteer plants in the previous season's corn field, maize is not considered as a weed in the U.S.

In a telephone conversation with Dr. Chester 'Chet' DeWald, USDA-ARS, Woodward, OK, a geneticist working on improvement of grasses, he stated that relatively few accessions of *T. dactyloides* will cross with maize and the majority of progeny are not fertile or viable even in those that do. In controlled crosses, if the female parent is maize, there is a greater likelihood of obtaining viable seed. When these hybrids have been backcrossed to maize in attempts to introgress *Tripsacum* genes for quality enhancement or disease resistance, the *Tripsacum* chromosomes are typically lost in successive generations. In many instances where hybridization has been directed between these two species, the resultant genome is lacking in most or all of the chromosomal complements of one of the parent species in subsequent generations.

Only recently has Dr. DeWald (or anyone else) succeeded in obtaining a true *Tripsacum* cytoplasm with a maize nuclear background. This was done by using gama grass as the female parent and maize as the male or pollen donor. Numerous accessions were tested and crosses made before this came to fruition. The *Tripsacum* derived mitochondrial chondrome and chloroplast plastome in these hybrids contribute to the seed qualities of the plants, but the nuclear genome appears to be totally maize in origin (DeWald *et al*., 1999).

Dr. DeWald concluded that the possibility of maize contributing genetic material to Eastern Gama Grass through random pollen flow in agricultural or natural situations is extremely remote based upon his experience trying to create hybrids under the best of conditions. He also felt that no other known grass species present in the continental U.S. would interbreed with commercial maize populations (*i.e.*, be recipients of pollen-directed gene flow). This is in agreement with Holm *et al*. (1979) who determined that none of the sexually compatible relatives of corn in the U.S. are considered to be serious, principal, or common weeds in the U.S.

c. *Zea* species - Teosintes - General Biology

Teosintes, specifically *Z. mays* ssp. *mexicana* (Schrader) Iltis, *Z. mays* ssp. *parviglumis* Iltis and Doebley*, Z. mays* ssp. *huehuetenangensis* (Iltis and Doebley) Doebley*, Z. luxurians* (Durieu and Ascherson) Bird*, Z. perennis* (Hitchc.) Reeves and Mangelsdorf and *Z. diploperennis* Iltis, Doebley and Guzman*,* have co-existed and co-evolved in close proximity to maize in the Americas over thousands of years; however, maize and teosinte maintain distinct genetic constitutions despite sporadic introgression (Doebley, 1990).

The teosintes retain a reduced cob-like fruit / inflorescence that shatters more than cultivated maize, but still restricts the movement of seeds as compared to more widely dispersed weedy species. Hence, the dispersal of large numbers of seeds, as is typical of weeds, is not characteristic of teosintes or maize. In their native habitat, some teosintes have been observed to be spread by animals feeding on the plants. Teosintes and teosinte-maize hybrids do not survive even mild winters and could not propagate in the U.S. corn belt. Additionally, some types have strict day length requirements that preclude flowering within a normal season (*i.e*., they would be induced to flower in November or December) and, hence, seed production under our temperate climate (Beadle, 1980; Iltis, personal communication; 2000; Wilkes, personal communication; 2000; Wilkes, 1967).

Since both teosinte and *Tripsacum* are included in botanical gardens in the U.S., the possibility exists (although unlikely) that exchange of genes could occur between corn and its wild relatives. The Agency is not aware, however, of any such case being reported in the United States. Gene exchange between cultivated corn and transformed corn would be similar to what naturally occurs at the present time within cultivated corn hybrids and landraces. Plant architecture and reproductive capacity of the intercrossed plants will be similar to normal corn, and the chance that a weedy type of corn will result from gene flow with cultivated corn is extremely remote.

Like corn, *Zea mays* ssp. *mexicana* (annual teosinte) and *Zea diploperennis* (diploid perennial teosinte) have 10 pairs of chromosomes, are wind pollinated, and tend to outcross, but are highly variable species that are often genetically compatible and interfertile with corn, especially when maize acts as the female parent. *Zea perennis* (perennial teosinte) has 20 pairs of chromosomes and forms less stable hybrids with maize (Edwards *et al*., 1996; Magoja and Pischedda, 1994). Corn and compatible species of teosinte are capable of hybridization when in proximity to each other. In Mexico and Guatemala, teosintes exist as weeds around the margins of corn fields. The F_1 hybrids have been found to vary in their fertility and vigor. Those that are fertile are capable of backcrossing to corn. A few isolated populations of annual and perennial teosinte were said to exist in Florida and Texas, respectively (USDA-APHIS, 1997). The Florida populations were presumably an escape from previous use of *Z. mays* ssp. *mexicana* as a forage grass, but local botanists have not documented any natural populations of this species for approximately twenty-five years (Keith Bradley, personal communication, 2000; David Hall, personal communication, 2000; Richard Wunderlin, personal communication, 2000).

Consultation with botanists and agronomists familiar with Texas flora suggested that no teosinte populations exist in the state (Benz, personal communication, 2000; Read, personal communication, 2000; Orzell, personal communication, 2000; Wilson, personal communication, 2000). Further, given the day length characteristics of *Z. diploperennis*, it is highly unlikely a sustaining population would result from introduction of this species. *Z. mays* ssp. *mexicana, Z. mays* ssp. *parviglumis, Z. luxurians* and *Z. diploperennis* may cross with maize to produce fertile hybrids in many instances (Wilkes, 1967). None of these teosinte species have, however, been shown to be aggressive weeds in their native or introduced habitats (John Schoper, personal communication, 1999). Except for special plantings as noted above, teosinte is not present in the U.S. or its territories. Its natural distribution is limited to Mexico, Honduras, Nicaragua, El Salvador and Guatemala.

Given the cultural and biological relationships of various teosinte species and cultivated maize over the previous two millennia, it would appear that significant gene exchange has occurred (based upon morphological characters) between these two groups of plants and that no weedy types have successfully evolved as a result. More recent cytogenetic, biochemical and molecular analyses have indicated that the degree of gene exchange is far less than previously thought (Doebley, 1984; Doebley *et al*., 1987; Kato, 1997a, 1997b; Smith *et al*., 1985). Partial and complete gametophytic incompatibility has been documented among cultivated maize, landraces and teosinte (Kermicle, 1997). The former is demonstrated by differential pollen growth and a skewed recovery of alleles linked to incompatibility genes. Complete incompatibility mechanisms serve to isolate a species or subspecies and are evidenced as pollen exclusion or non-functioning of pollen types on certain genotypes. Attempts to cross six collections of *Zea mays* ssp. *mexicana* with U.S. maize cultivars (W22, W23) yielded no or few seeds in five of the six groups (Kermicle and Allen, 1990).

Based on the ability of maize to hybridize with some teosintes, the suggestion of previous genetic exchange amongst these species over centuries, and their general growth habits, any introgression of genes into wild teosinte from *Zea mays* is not considered to be a significant agricultural or environmental risk. The growth habits of teosintes are such that the potential for serious weedy propagation and development is not biologically plausible in the United States.

d. Conclusions

The potential for pollen-directed gene flow from maize to Eastern Gama Grass is extremely remote. This is evidenced by the difficulty with which *Tripsacum dactyloides* x *Zea mays* hybrids are produced in structured breeding programs. Additionally, the genus does not represent any species considered as serious or pernicious weeds in the United States or its territories. Any introgression of genes into this species as a result of cross fertilization with genetically-modified maize is not expected to result in a species that is weedy or difficult to control. In many instances where hybridization has been directed between these two species, the resultant genome is lacking in most or all of the maize chromosomal complement in subsequent generations.

Many of the *Zea* species loosely referred to as "teosintes" will produce viable offspring when crossed with *Zea mays* ssp. *mays*. None of these plants are known to harbor weedy characteristics and none of the native teosinte species, subspecies or races are considered to be aggressive weeds in their native or introduced habitats. In fact, many are on the brink of extinction where they are indigenous and will be lost without human intervention (*i.e*., conservation measures). Further, none of the landraces or cultivated lines of *Zea mays* are considered to have weedy potential and are generally considered to be incapable of survival in the wild as a result of breeding practices (*i.e*., selection) during domestication of the crop.

C. Endangered Species Considerations

Cry3 proteins including Cry3Bb1 are known to be highly specific against coleopteran insects and are not hazardous to vertebrate animals. It has been generally demonstrated that Cry3 proteins do not pose a hazard to non-target animals or invertebrates. The Cry3Bb1 protein appears to be specifically toxic to Chrysomelid beetles including corn rootworms (*Diabrotica* spp.) and Colorado potato beetles (*Leptinotarsa decemlineata*) (MRID No. 455328-07). Currently, there are no Chrysomelid species listed on the endangered species list and no other species are known to be sensitive to Cry3Bb1. Therefore, no adverse affects from Cry3Bb1 event MON 863 are expected against endangered species. Nevertheless, all endangered/threatened beetle species habitats found in the counties where corn is grown were examined to determine possible exposure to corn pollen. Their habitat (and breeding grounds) were found not to overlap with corn fields. Therefore no endangered beetles will be exposed to potentially harmful levels of corn tissue or pollen containing Cry3Bb1 protein.

Terrestrial and aquatic exposure were considered in this assessment since non-target coleopterans may be exposed to the Cry3Bb1 protein within corn fields or in surrounding areas from plant tissue (e.g., pollen) movement offsite. However, the distance pollen moves outside of the corn field should be considered. Published data show that less than 25 grains of pollen per cm^2 are expected 4-5 meters from the corn field edge. A relative comparison of surface ratio of milkweed to other substrates (e.g., other host plants, arthropod prey, animal carrion) can be used as a basis for estimating the amount of pollen that may leave the field. The maximum concentration of Cry3Bb1 protein has been determined to be 93 µg/g fresh weight pollen. Based on this concentration, <0.03 µg Cry3Bb1 protein/g of diet would be expected to be deposited 4-5 meters from the field edge. The potential of aquatic organisms to be exposed to the Cry3Bb1 protein is minimal. Such exposure would occur from runoff of the protein (either free or sequestered in plant debris) into adjacent water bodies or pollen drift. Since movement of Cry3Bb1 in soil into water bodies is expected to be negligible, pollen drift was considered the primary source of potential hazard to endangered aquatic Coleoptera. According to estimates based on published studies, if 100% of the pollen grains leaving the field were deposited in a 1 ha pond with 2 m depth and located ≥ 1 m from the edge of the corn field, <0.0001 µg

Cry3Bb1/mL of water would be expected. This is a few orders below the toxic level to any insect.

Many of the endangered and threatened beetles occur in cave or aquatic habitats. None of these endangered beetles are expected to occur in or near corn fields. The American burying beetle may occur in old fields or cropland hedge rows. However, based upon the feeding habits of the American burying beetle, the beetle is not expected to occur within corn fields nor will it be exposed to Cry3Bb1 protein. Adult American burying beetles are classified as opportunistic scavengers that feed on anything dead and bury vertebrate carcasses on which their larvae feed. Carrion regurgitated by adults is fed to larvae until they are able to feed directly on a carcass.

In addition, Monsanto conducted a hazard assessment, exposure assessment and risk characterization to demonstrate that Cry3Bb1 does not pose a risk to endangered Coleoptera (MRID No. 455770-03). This endangered species assessment was based on the Hazard Evaluation Division, Standard Evaluation Procedure, Ecological Risk Assessment (U.S. EPA, 1986). The Agency reviewed this assessment and found it acceptable.

An examination of the endangered bird and bat species shows that their breeding habitats are mostly non-agricultural. Insectivorous bats do not prey on larvae. They rely on flying insects. Taking these, and other pertinent issues into consideration, it becomes apparent that reduction in the target pests of corn would not have an effect on the food source of endangered birds and bats. Of those that do encroach on agricultural fields in the rare instances where these species may feed on the target pests, the reduction in the pest species will merely cause them to rely on other plentiful insects as a source of food. Submitted and published field data reviewed in this document show that a wide variety of insects remain abundant in Cry3Bb1 corn fields as opposed to non-Bt fields when conventional insect pest control practices are used. Therefore the data show that Bt crops should actually be beneficial to bird and bat populations.

The reviewed non-target data confirm the expectation that Cry3Bb1 corn will have no adverse effect on endangered and/or threatened species listed by the US Fish and Wildlife Service, including mammals, birds or terrestrial and aquatic plants and invertebrate species. Therefore, no consultation with the USFWS is required under the Endangered Species Act,

D. Environmental Assessment Summary:

The Agency is using a Maximum Hazard Dose Tiered system for biopesticide non-target wildlife hazard assessment. When no adverse effect at the maximum hazard dose are observed, the Agency concludes that there are no unreasonable adverse effects from the use of the pesticide. From all of the required and voluntarily developed indicator and host range species test data on Cry3Bb1 corn, including the supplementary two year field data, the Agency concludes that the levels of Cry3Bb1 protein in corn will not pose unreasonable adverse effects to corn field flora and fauna. Available data also indicate that there should be minimal short term accumulation of

Cry3Bb1 protein in agricultural soil. In addition, no adverse effect on listed endangered and threatened species listed by the US Fish and Wildlife Service is expected from the proposed Cry3Bb1 CRW resistant corn registration.

At present, the Agency is aware of no identified significant adverse effects of Cry3Bb1 proteins on the abundance of non-target beneficial organisms in any population in the field, whether they are pest parasites, pest predators, or pollinators. Field testing and field census data submitted to the Agency show minimal to undetectable changes in the beneficial insect abundance or diversity. In corn fields densities of predatory and non-target insects are generally higher on Cry3Bb1 corn than non-Bt corn primarily because the Cry3Bb1 corn is not subjected to the same number of applications of nonspecific pesticides. Two year invertebrate abundance studies do not show a shift in the biodiversity in Cry3Bb1 corn, except in cases where the predators are dependent on the pest insect as prey. In contrast, treatment with chemical pesticides, when studied, had significant effects on the total numbers of insects and on the numbers within the specific groups. To date the available field test data show that compared to crops treated with conventional chemical pesticides, the transgenic crops have no detrimental effect on the abundance of non-target insect populations. However, annual insect monitoring of representative commercial fields will continue for long term biodiversity effects assessment.

The Agency believes that cultivation of Cry3Bb1 corn may result in fewer adverse impacts to non-target organisms than result from the use of chemical pesticides. Under normal circumstances, Cry3Bb1 corn requires substantially fewer applications of chemical pesticides. This should result in fewer adverse impacts to non-target organisms because application of nonspecific conventional chemical pesticides is known to have an adverse effect on non-target beneficial organisms found living in the complex environment of an agricultural field. Many of these beneficial organisms are important integrated pest management controls (IPM) for secondary pests such as aphids and leafhoppers. The overall result of cultivation of corn expressing Cry3Bb1 proteins is that the number of chemical insecticide applications for non-target pest control is reduced for management of multiple pest problems.

The movement of transgenes from Cry3Bb1 host plant into weeds and other crops has also been considered. The Agency has determined that there is no significant risk of gene capture and expression of Cry3Bb1 protein by wild or weedy relatives of corn in the U.S., its possessions or territories. The fate of Cry3Bb1 protein in soils and indirect effects on soil biota have also been evaluated. Test data show that most of the Cry protein deposited into soil is quickly degraded, although a residual amount may persist in biologically active form for a much longer period of time. It is also reported that the same degree of Bt Cry protein persistence takes place in soils that have been exposed to repeat Bt spray applications when compared to soil exposed to growing Bt crop. Limited data do not indicate that Cry proteins have any measurable effect on microbial populations in the soil. Horizontal transfer from transgenic plants to soil bacteria has not been demonstrated. Published studies of Bt Cry protein in soil show no effect on bacteria, actinomyces, fungi, protozoa, algae, nematodes, springtails or earthworms. In addition, new plants planted in Bt Cry protein containing soil do not take up the Bt protein.

Conclusions:

This reassessment finds no hazard to the environment at the present time from cultivation of Cry3Bb1 protein expressing corn for a time-limited registration.

E. Public Comments

Public comments expressing specific environmental concerns received in response to the FR Notice announcing the receipt of Monsanto application are being addressed in a separate document.

F. Supplemental Studies Needed for Long Term Cry3Bb1 non-target Hazard Assessment

The Agency has sufficient information to believe that there is no risk from the proposed uses of Cry3Bb1 corn to non-target wildlife, aquatic and soil organisms. However, after consultation with the FIFRA Scientific Advisory Panel in August, 2002 and from several public comments, the Agency is requesting additional data. The supplementary studies would provide additional weight to support the Agency's conclusions. Therefore, the Agency is requesting the following data (Table 5) to ascertain any possible adverse environmental effects from long term use of this product, as well as testing on more appropriate non-target invertebrates found in corn fields. The Agency does not believe that this data requirement was reasonably foreseeable by the applicant at the time of application.

Table 5. Supplemental data:

Testing Category	Type of Data
Avian chronic exposure testing	The submitted avian dietary toxicity data are not sufficient to make a final avian hazard assessment from repeated exposure(s) to higher doses of Cry3Bb1 corn. A six week broiler dietary study with 60% - 70% MON 863 corn in the diet is needed to assess hazard to wild and domesticated fowl from chronic exposure to high levels of Cry3Bb1 protein.
Non-target insect more appropriate for corn fields	Conduct a maximum hazard dose laboratory toxicity test with *Orius insidiosus* (minute pirate bug).
Non-target insect more appropriate for corn fields	Conduct a maximum hazard dose laboratory toxicity test with a carabid (ground beetle).
Non-target insect more appropriate for corn fields	Maximum hazard testing with *Tetraopes* (red milkweed beetle) should be performed because they are a more logical choice than the monarch butterfly
Field community effects	Submit final results to field studies previously summarized in MRID No.456530-03. The carabid and nematode data are of particular interest.

Testing Category	Type of Data
Ecosystem effects	Additional long range field studies should also be conducted based on recommendations of the August, 2002 SAP found in the conclusion section of the review of MRID No. 457916-01 above.
Soil fate studies	Additional long range soil degradation field studies should also be conducted including the parameters outlined by the August 2002 SAP MRID No. 457571-02 above

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